IN VIVO AND IN VITRO RESPONSES OF CATTLE TO PROSTAGLANDIN $F_{2\alpha}$

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By

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Dairy heifers (n=37) were treated twice with 33.5 mg prostaglandin $F_{2\alpha}$ tham salt ($PGF_{2\alpha}$) 12 days apart to determine if this management scheme increased percentage of animals expressing estrus after the second injection. Average plasma progestin concentrations were 3.33 and 6.51 ng per ml at the first and second injection. A greater percentage of heifers expressed estrus after the second injection (89 vs 60%). Consequently, treatment of cattle two times, 12 days apart, is a management scheme that increases percentage of animals undergoing corpus luteum regression for subsequent insemination.

To determine if a luteolytic dose of $PGF_{2\alpha}$ exerted any physiological effects that may detract from its practical usefulness, an experiment was conducted to determine if heart rate, blood pressure, arterial blood temperature and uterine temperature change after $PGF_{2\alpha}$ injection. To

monitor these responses a polyvinyl catheter and a thermocouple were surgically placed into the external iliac artery and an additional thermocouple placed into the uterine serosa of two cows.

In response to intramuscular injections (IM) of either 33.5 mg $PGF_{2\alpha}$ (n=3) or saline (n=3), there was a slight rise in mean blood pressure and heart rate. No major alterations in blood or uterine temperatures occurred over 3 hr following $PGF_{2\alpha}$ injection.

Following 2 min intravenous infusion of $PGF_{2\alpha}$ (33.5 mg; n=2), blood pressure increased from 140 mm Hg to 230 mm Hg, whereas heart rate decreased from 34 to 16 beats per 30 sec. Associated with these changes were an increase in uterine temperature and a concurrent widening in the temperature difference between the uterus and arterial blood. These results are suggestive of a marked decrease in uterine blood flow. A study utilizing electromagnetic blood flow transducer probes demonstrated that $PGF_{2\alpha}$ (8 mg; intramuscularly) decreased uterine blood flow (72 to 41 ml per min) in estradiol primed (20 µg), ovariectomized sheep (n=3).

Experiments indicated that a luteolytic dose of $PGF_{2\alpha}$ administered IM caused no major alterations in the physiological parameters measured, whereas intravenous infusion caused major alterations in circulatory homeostasis.

An in vitro incubation system was developed to study bovine ovarian follicular steroidogenesis. Follicles were obtained from cows treated twice daily with FSH-p (Armour Baldwin Labs) on days 16 to 19 of the estrous cycle. After follicle dissection from the ovary, follicular fluid was aspirated and follicles incubated individually for 14 hr during which the medium was changed every 2 hr. Hormonal treatments were added to the medium, and treatment effects analyzed by least squares analyses. Incubation medium (100 ml) consisted of 80% Medium 199 with Hanks salts, 20% fetal calf serum, 5.6 mg insulin, 5.6 mg ascorbic acid and 1.8 mg gentamicin.

Follicles (n=5) incubated for 14 hr secreted four times more estradiol into the medium than extracted from unincubated frozen follicles (n=5; $P<.01$). In Experiment 2, $PGF_{2\alpha}$ (5 ng per ml medium) and LH (50 ng per ml medium) had no effect on in vitro estradiol secretion. Progestin secretion increased during incubation in control follicles and this increase was stimulated by LH ($P<.01$). In control and $PGF_{2\alpha}$ treated follicles testosterone secretion increased in the pretreatment periods and decreased gradually during treatment periods. However, LH treatment stimulated testosterone secretion ($P<.01$).

$PGF_{2\alpha}$ added to medium at doses of 5, 100 and 1000 ng per ml had no effect on estradiol secretion in Experiment 3. Similarly, FSH (100 ng per ml), testosterone (5×10^{-7}M) or combination of both had no effect on estradiol secretion in Experiment 4.

Histological examination of incubated follicles suggested that this nonresponsiveness of estradiol secretion may be due to disassociation of granulosa cells from the basal lamina into the antrum.

INTRODUCTION

Major limitations to artificial insemination in cattle are failure to detect estrus and improper timing of insemination. In recent years there has been considerable interest in establishing an ovulation control system which includes a predetermined time for insemination. Optimally, such a system would eliminate the need for estrous detection and provide a precise time for insemination which maximizes conception rates. Such reproductive management programs would be of paramount importance in the Florida dairy industry where large herd sizes hamper reproductive management efficiency.

Recent studies indicated that prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) may be used for ovulation control in cattle. $PGF_{2\alpha}$ induces luteal regression which is followed by ovulation; however, it is not effective during the first 5 days of the estrous cycle. Therefore, its general usefulness as a practical tool for ovulation control is limited unless alternative management systems are developed. Very few studies have been conducted in cattle to determine if $PGF_{2\alpha}$ has any detrimental physiological effects which may limit its general usefulness.

Objectives of the first series of experiments were twofold:

1. to test a dual injection management system for $PGF_{2\alpha}$ administration. This system was designed to eliminate animals from being in the nonresponsive first 5 days of the estrous cycle at the second $PGF_{2\alpha}$ administration.
2. to determine if $PGF_{2\alpha}$ affects heart rate, mean arterial blood pressure, arterial blood temperature and uterine temperature when administered in a luteolytic dose to dairy cattle.

$PGF_{2\alpha}$ also has been implicated as a natural luteolytic agent for luteal regression in several species and to affect estradiol secretion in cattle. In a second series of experiments an incubation system for bovine ovarian follicles was developed to study the effects of $PGF_{2\alpha}$ and gonadotropins on follicular estradiol secretion in vitro. Results from these studies may contribute to our understanding of follicular steroidogenesis and corpus luteum regression. These phenomena must be understood before luteal regression, follicular maturation and ovulation can be controlled optimally.

SECTION I

LITERATURE REVIEW

Prostaglandins

Prostaglandins (PG) are unsaturated 20 carbon fatty acids containing a cyclopentane ring and two aliphatic side chains. This class of compounds is widely distributed in animal tissues and has powerful pharmacological effects in many biological systems. Recent findings indicate that PGs are produced physiologically and may act as local hormones or autocoids.

Luteolytic Effect of Prostaglandin $F_{2\alpha}$

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) has various physiological effects, and one of these is its lytic action on the corpus luteum. $PGF_{2\alpha}$ induces luteal regression in many species, including bovine, ovine and equine, and this response has stimulated the interest of animal scientists. Inskeep (1973) reviewed the luteolytic properties of $PGF_{2\alpha}$ in domestic animals, and they have been well documented in the bovine (Chenault et al., 1976; Hafs et al., 1974, Thatcher and Chenault, 1976).

In cattle, injection of $PGF_{2\alpha}$ is followed by a sequence of hormonal patterns very similar to those observed during spontaneous corpus luteum regression, follicular maturation and ovulation. Following a single administration of $PGF_{2\alpha}$, plasma progestins decline rapidly reaching estrus concentrations by 24 hr posttreatment, whereas estradiol concentrations slowly increase and stimulate an ovulatory surge of luteinizing hormone (LH) at 79$\pm$ 21 hr ($\bar{X}\pm$S.D.) posttreatment. Ovulation occurs at 99.5$\pm$ 19 hr after $PGF_{2\alpha}$ administration (Chenault et al., 1976). Various workers have demonstrated that fertility after the post-$PGF_{2\alpha}$ ovulation is comparable to fertility after spontaneous ovulations in untreated herdmates (Ellicott et al., 1974; Lambert et al., 1975; Lauderdale et al., 1974; Tobey and Hansel, 1975). Lauderdale et al. (1974) also demonstrated that fertility in animals bred twice at 72 and 90 hr post-$PGF_{2\alpha}$, regardless of estrus, was comparable to fertility in both untreated controls and $PGF_{2\alpha}$ treated animals bred at the posttreatment estrus.

$PGF_{2\alpha}$ induction of luteal regression followed by ovulation and normal fertility provides the minimal requirements for ovulation control. However, there are several factors associated with use of a single injection of $PGF_{2\alpha}$ which limits its practical usefulness for ovulation control. It is well documented that $PGF_{2\alpha}$ is effective only after day 5 of the estrous cycle (Cooper, 1974; Lauderdale, 1972;

Henricks et al., 1974) when a functional corpus luteum is present. Therefore on any random day of injection, assuming a 21 day cycle and all animals are cycling, only a 76% maximum animal response would be expected. The other 24% would be in the nonresponsive first 5 days of the estrous cycle.

To minimize the number of animals in this nonresponsive stage of the estrous cycle (first 5 days), Lauderdale et al. (1974) treated only animals with a palpable corpus luteum and treated all others 1 week later. Despite this precaution only 65% of the treated animals (n=239) displayed visual signs of estrus in the 7 days following $PGF_{2\alpha}$ administration. This can be compared to an 80% level of estrous detection in untreated herdmates during a concurrent 18 to 25 day interval. Of the animals expressing estrus in the $PGF_{2\alpha}$ treated group, 90% of these heats were distributed over a 3 day period. Similar variation in onset of estrus following $PGF_{2\alpha}$ treatment has been reported by Chenault et al. (1976), Louis, Hafs and Sequin (1973) and Louis, Hafs and Morrow (1974a). These results indicate that management schemes must be developed which manipulate the estrous cycle so that all animals treated respond to $PGF_{2\alpha}$. Furthermore, the variability in time of estrus and/or ovulation after $PGF_{2\alpha}$ treatment must be reduced if such ovulation control systems are to include a timed insemination component after $PGF_{2\alpha}$ injection.

Several management schemes have been utilized to overcome the problem of nonresponse during the first 5 days of the estrous cycle. Lambert et al. (1975) bred all animals which came into estrus over a 4 day period and then treated all remaining animals with $PGF_{2\alpha}$. This treatment scheme would eliminate animals from being in the nonresponsive stage of the cycle on the day of $PGF_{2\alpha}$ treatment. Another method would be to treat all animals with a progestogen for 5 to 7 days and then administer $PGF_{2\alpha}$. The short-term progestogen treatment would suppress estrus and ovulation in animals whose corpora lutea regressed during the progestogen treatment. These animals would return to estrus upon withdrawal of the progestogen block. Cows that had corpora lutea at the end of progestogen treatment would return to estrus in response to $PGF_{2\alpha}$ whereas those animals having developing corpora lutea (days 1 to 5) at the beginning of progestogen treatment also would have responsive corpora lutea to $PGF_{2\alpha}$. This technique has been used for ovulation control, and the induced ovulations are followed by normal fertility (Heersche et al., 1974; Roche, 1976a).

An alternate to these management schemes is to treat all animals twice with $PGF_{2\alpha}$, 10 to 12 days apart, as suggested by Inskeep (1973). Theoretically, this would increase the potential percentage of animals in the responsive stage of the cycle at the second injection. All animals in the

responsive stage of the cycle, days 6 through 21, at the first day of $PGF_{2\alpha}$ injection will express an estrus and be in the responsive luteal stage of the cycle at the second injection, 10 to 12 days later. All animals in the nonresponsive stage of the cycle, days 1 to 5, at the first injection will not respond to $PGF_{2\alpha}$ and will be in a responsive luteal stage at the second injection. Using this dual injection technique with an ICI analogue to $PGF_{2\alpha}$ (ICI 80,996), Cooper (1974) reported that only 2 of 175 animals failed to respond to the second $PGF_{2\alpha}$ treatment. Furthermore, 90% of the animals were in estrus between 48 and 72 hr after the second treatment and fertility of this second estrus was normal. In a large field study using this technique, Hafs, Mans and Lamming (1975) reported sufficient synchronization of ovulation after the second $PGF_{2\alpha}$ injection to obtain fertility from one insemination at 80 hr after $PGF_{2\alpha}$ comparable to two timed inseminations at 70 and 80 hr. Furthermore, fertility in both timed insemination groups was equivalent to that of untreated herdmates. This large field study indicated that treatment with $PGF_{2\alpha}$ two times, 10 to 12 days apart, improved potential responsiveness and precision of synchronization to allow for a single timed insemination after the second injection of $PGF_{2\alpha}$. A large field study by Cooper and Jackson (1975) failed to support these results concerning a single fixed time of insemination. Fertility

to a single insemination at 72 or 80 hr after the second $PGF_{2\alpha}$ treatment was 7% lower than fertility in untreated controls or cattle bred twice at 72 and 96 hr after the second injection. Both groups of workers agreed, however, that there was equal fertility between controls and animals bred twice.

Increased fertility to a single timed insemination might be accomplished by controlling time of ovulation. An LH surge is initiated in cattle by synthetic or natural gonadotropin releasing hormones (Kalra et al., 1974; Zolman et al., 1973) or estradiol (Hobson and Hansel, 1972; Short et al., 1973). Injection of gonadotropin releasing hormone (GnRH, a synthetic releasing hormone) at 48 or 60 hr after $PGF_{2\alpha}$ resulted in significantly higher plasma LH concentrations than GnRH given at 0, 12 or 24 hr. However, plasma progestins also were elevated following GnRH at 0, 12, 24 and 48 but not 60 hr after $PGF_{2\alpha}$ (Kalra et al., 1974). Rodriguez et al. (1975) reported that GnRH given at 48 hr after $PGF_{2\alpha}$ injection reduced significantly the frequency of estrous behavior. It is possible that elevated plasma progestins or premature ovulation induced by GnRH suppressed estrus in these animals. These two studies suggested that GnRH should be administered no sooner than 60 hr after $PGF_{2\alpha}$. Graves et al. (1975) reported that GnRH given at 60 hr after $PGF_{2\alpha}$ was effective in reducing variation in ovulatory time.

Welch et al. (1975) reported that injection of estradiol benzoate (400 μg) at 48 hr after $PGF_{2\alpha}$ reduced variability in onset of estrus during the period of 56 to 88 hr post-$PGF_{2\alpha}$. However, no advantage in fertility was seen in the estradiol treated animals. No workers have reported an advantage in fertility rate when $PGF_{2\alpha}$ was used in combination with an LH releaser as compared to $PGF_{2\alpha}$ treatment alone (Graves et al., 1975; Tobey and Hansel, 1975; Welch et al., 1975).

Differences in reports (Cooper and Jackson, 1975; Hafs et al., 1975) as to whether fertility at a single timed insemination is comparable to that obtained with two timed inseminations indicates that optimal management systems for the use of $PGF_{2\alpha}$ have not been fully developed and additional studies are warranted.

The mechanism by which $PGF_{2\alpha}$ initiates or induces corpus luteum regression is unknown. Pharriss and Wyngarden (1969) suggested that $PGF_{2\alpha}$ exerted a venoconstricting effect on the ovarian vein which resulted in regression by anoxia. Several authors since have disproved this theory by demonstrating that the decline in plasma progesterone after $PGF_{2\alpha}$ could not be correlated with any change in total ovarian blood flow (Behrman, Yoshingaga and Greep, 1971; McCracken, Baird and Goding, 1971). However, Novy and Cook (1973) and Thorburn and Hales (1972) demonstrated that $PGF_{2\alpha}$ may redistribute intra-ovarian blood flow. Using microsphere techniques these workers reported that blood flow to the corpus luteum was reduced, whereas blood flow to the stroma and

follicular component of the ovary was increased, following $PGF_{2\alpha}$ administration.

Morphological electron microscopic studies have indicated that $PGF_{2\alpha}$ induced regression of the corpus luteum is comprised of functional followed by structural regression. Functional regression is the termination of progesterone secretion, whereas structural regression is the physical destruction of the luteal cell. Furthermore, $PGF_{2\alpha}$ induced regression of sheep corpora lutea mimics structural and functional sequences characteristic of normal luteal regression (Stacy, Gremmell and Thorburn, 1976). Lowered plasma progestin concentrations were observed at 3 hr postinfusion of a luteolytic dose of $PGF_{2\alpha}$ and occurred prior to any noticeable disorganization of cell structure. However, by 6 hr postinfusion cellular signs of early regression were present, and by 24 hr gross signs were observed. Early signs of regression included lack of secretory granules and accumulation of lipid droplets within the cells. Following infusion of low doses of $PGF_{2\alpha}$ plasma progestin levels decreased for a short period of time. These decreases were associated with early signs of functional regression; however structural regression did not occur.

Because structural regression always was preceded by accumulation of lipid droplets, Stacy et al. (1976)

postulated that "During regression $PGF_{2\alpha}$ may inhibit one or more stages in steroidogenesis so that lipid, being diverted from its normal synthetic pathway, accumulates as droplets of cholesterol ester in the luteal cell" (p.290). Umo (1975) also using electron microscopy observed functional regression prior to any evidence of structural regression following intramuscular injection of $PGF_{2\alpha}$ in sheep. These results clearly demonstrated that $PGF_{2\alpha}$ does not cause termination of progesterone secretion (functional regression) by mass destruction of the luteal cells, but most likely acts on steroidogenic and other biochemical pathways. Structural regression follows and may be a consequence of these biochemical changes. It has been demonstrated that $PGF_{2\alpha}$ increases lysosomal fragility and this may be the mechanism by which $PGF_{2\alpha}$ induces structural regression (Weiner and Kaley, 1972).

Henderson and McNatty (1975) presented a biochemical hypothesis by which $PGF_{2\alpha}$ may initiate corpus luteum regression. LH binds to cell membranes of luteal cells (Channing and Kammerman, 1974) and has a stimulatory effect on progesterone synthesis. This response is mediated through activation of the adenylate cyclase system in the cell membrane to produce adenosine 3', 5'-monophosphate (CAMP; Savard, Marsh and Rice, 1965) which acts as an intracellular second messenger to stimulate progesterone synthesis.

Henderson and McNatty hypothesized that $PGF_{2\alpha}$ binds to the membrane of the luteal cell and directly or indirectly prevents transmission of the activating signal from the LH coupling component to the adenylate cyclase catalytic site. This would prevent synthesis of CAMP and thereby inhibit progesterone synthesis. They also suggested that $PGF_{2\alpha}$ may not be effective during the first 5 days of the estrous cycle because LH from the ovulatory surge saturates the regulatory units of the luteal cells, and this bound hormone protects the young cells. Therefore, $PGF_{2\alpha}$ would only be luteolytic when sufficient LH molecules have been dissociated from receptors and thus have exposed the receptors to the action of $PGF_{2\alpha}$.

An alternate explanation for the ineffectiveness of $PGF_{2\alpha}$ during the first 5 days of the estrous cycle can be postulated. Specific receptors for $PGF_{2\alpha}$ have been demonstrated in the bovine corpus luteum (Kimball and Lauderdale, 1975; Rao, 1975) and preliminary data indicated that there were fewer $PGF_{2\alpha}$ receptors on day 4 than on days 8, 12, 14 or 16 (Kimball et al., 1976). These data suggested that $PGF_{2\alpha}$ may not be effective until specific $PGF_{2\alpha}$ receptors have been synthesized in the newly formed corpus luteum.

To date, the mechanisms by which $PGF_{2\alpha}$ induces luteal regression and the reasons for $PGF_{2\alpha}$ being ineffective during the first 5 days of the cycle are unknown. Additional

studies in this area are essential to further our understanding of these phenomena. Answers to these questions may be useful in designing ovulation control systems and may increase our understanding of natural luteal regression.

Pharmacological Effects of $PGF_{2\alpha}$

$PGF_{2\alpha}$ exerts many biological effects other than induction of luteal regression. These effects range from its endocrine (Louis et al., 1974b) and previously mentioned effects on the reproductive system to its potent smooth muscle stimulatory action (Main, 1973; Nakano and Cole, 1969).

Plasma prolactin and growth hormone were increased within 10 min and glucocorticoids within 30 min in dairy heifers, following intramuscular (IM) injection of various $PGF_{2\alpha}$ doses, within the luteolytic range (15 to 60 mg). No changes were observed in plasma insulin or free fatty acids following intravenous (IV) injection (5 mg) or infusion (15 mg at .5 mg/minute) of $PGF_{2\alpha}$, whereas plasma glucose was elevated following IV administration (Louis et al., 1974b). Plasma LH also was increased following IM injection, but the interval to the increase (1.5 to 6 hr) and the amount of increase (2.7 to 17 ng per ml) was highly variable among animals. More recently this increase has been attributed to the removal of negative inhibition by progesterone on the hypothalamo-pituitary axis rather than a

direct stimulatory effect of $PGF_{2\alpha}$ on LH release (Louis, Stellflug and Hafs, 1975). Lauderdale et al. (1975) observed an increase in plasma catecholamines following IM administration of $PGF_{2\alpha}$ in mares.

$PGF_{2\alpha}$ also may affect estradiol concentrations in the bovine. Chenault et al. (1976) observed spikes of estradiol in peripheral plasma 2 to 9 hr following IM injection of a luteolytic dose (33.5 mg) of $PGF_{2\alpha}$ tham salt. Hixon et al. (1973) observed elevated peripheral plasma estrone and estradiol following intraluteal injection of $PGF_{2\alpha}$. Louis et al. (1974a) failed to detect any increase in estradiol secretion immediately following administration of $PGF_{2\alpha}$. $PGF_{2\alpha}$ may directly stimulate follicular estrogen synthesis, as Shemesh and Hansel (1975) reported that $PGF_{2\alpha}$ stimulated testosterone secretion by bovine follicular slices.

Effects of $PGF_{2\alpha}$ on peripheral blood pressure depend on the species of interest. Intravenous injection of $PGF_{2\alpha}$ causes a fall in arterial blood pressure in rabbits and cats (Horton and Main, 1964: Änggård and Bergström, 1963), whereas it has a pressor action in dogs (Nakano and McCurdy, 1968) and rats (DuCharme, Weeks and Montgomery, 1968; Viguera and Sunahara, 1969). Intravenous infusion causes constriction of metarterioles of the mesocecum and cremaster muscles in rats (Viguera and Sunahara, 1969). However, DuCharme et al. (1968) suggested the pressor action of $PGF_{2\alpha}$ in rats is

mediated by venoconstriction which is supported by the observation of Mark et al. (1971) that $PGF_{2\alpha}$ induced constriction of the saphenous vein in vitro. On the other hand, Nakano and Cole (1969) concluded that $PGF_{2\alpha}$ induced a vasoconstriction in the regional arteries which resulted in increased arterial pressure in dogs.

$PGF_{2\alpha}$ does not appear to have a direct action on the heart, as $PGF_{2\alpha}$ had no effect on force or rate of contraction in isolated chicken (Horton and Main, 1967) or hamster hearts (Lee et al., 1965). Neither did $PGF_{2\alpha}$ affect heart rate when injected into the canine sinus node artery (Nakano, Chiba and Nakajima, 1971). Therefore, changes in heart rate observed in several species following $PGF_{2\alpha}$ treatment must be an indirect effect, possibly through baroreceptor action, as $PGF_{2\alpha}$ does affect arterial blood pressure.

The gastroinestinal tract is stimulated by $PGF_{2\alpha}$. $PGF_{2\alpha}$ stimulates constriction of circular and longitudinal muscles of the ileum and colon in guinea pig, rat and man (Bennett and Fleshler, 1970) as well as the dog colon (Vanasin et al., 1970).

Uterine contractions are stimulated by $PGF_{2\alpha}$ in the ewe (Rexroad and Barb, 1975), mare (Capraro et al., 1976), rat, guinea pig (Horton and Main, 1964, 1967) and human (Karim et al., 1971).

The mechanism by which $PGF_{2\alpha}$ affects smooth muscles appears to be a direct action. Its effect on gastrointestinal smooth muscle is not through neural stimulation as these effects are not affected by parasympatholytic (antimuscarinic) drugs or alpha or beta blocking agents (Main, 1973). Several workers have demonstrated that $PGF_{2\alpha}$ may affect smooth muscle contractibility through control of intracellular calcium. $PGF_{2\alpha}$ stimulates release of calcium from the scarcoplasmic reticulum into the cell and inhibits ATP dependent calcium binding for the reuptake of calcium by the scarcoplasmic reticulum (Carsten, 1972; Coceani et al., 1969). These two actions of $PGF_{2\alpha}$ would increase the availability of intracellular calcium which is essential for muscular contraction.

Several workers also have indicated a role for $PGF_{2\alpha}$ in thermoregulatory mechanisms. Hales et al. (1973) demonstrated this action in a series of experiments in cool, thermoneutral and warm environments. $PGF_{2\alpha}$ injected into the lateral cerebral ventricle in sheep stimulated brain pathways involved in the stimulation of heat production and vasomotor tone, and inhibited heat loss pathways. Observations following $PGF_{2\alpha}$ injection included increased rectal temperature, decreased skin temperature (vasoconstriction) and decreased respiratory rate.

Intramuscular injection of $PGF_{2\alpha}$ in mares was followed by increased plasma catecholamines, sweating and reduced rectal temperature, whereas injection of epinephrine was followed by sweating, shivering and no decrease in rectal temperature (Lauderdale et al., 1975; Miller, Lauderdale and Geng, 1976). These results indicate that $PGF_{2\alpha}$ caused release of epinephrine which resulted in sweating. However, $PGF_{2\alpha}$ also appeared to inhibit heat production mechanisms (such as shivering) as evidenced by the drop in rectal temperature following $PGF_{2\alpha}$. This effect is opposite the heat producing and conserving effects of $PGF_{2\alpha}$ when administered into the lateral ventricles of sheep (Hales et al., 1973).

Reports on the pharmacological effects of $PGF_{2\alpha}$ on cattle are very limited and generally restricted to work with calves except for the endocrine studies mentioned previously. Lewis and Eyre (1972) reported that $PGF_{2\alpha}$ increased systemic blood pressure, pulmonary arterial pressure, abdominal venous pressure, respiratory volume and heart rate in calves. Aitken and Sanford (1975) also reported increased systemic blood pressure and heart rate, but observed a decrease in respiratory minute volume in response to $PGF_{2\alpha}$ in calves.

With the increased interest in the use of $PGF_{2\alpha}$ as a basis for an ovulation control system in cattle, additional research on the pharmacological effects of $PGF_{2\alpha}$ is warranted

to determine if $PGF_{2\alpha}$ has any detrimental biological effects which may limit its usefulness in cattle.

Follicular Steroidogenesis

Follicles generally are regarded as the primary source of preovulatory estrogens (Hisaw, 1947). However, beyond this point there is considerable debate in regard to tissues and pathways involved in estrogen biosynthesis. There appears to be considerable species variability and contradictory evidence within species to stimulate this debate.

Ovaries of cattle contain primary, secondary, tertiary and mature Graafian follicles. Larger follicles consist of ovum, granulosa cells and theca cells whereas primary follicles consist of the oogonia surrounded by a single layer of granulosa cells. Follicles are classified as secondary follicles when the zona pellucida has been formed around the cell membrane of the ovum and granulosa cells have multiplied into several layers. The tertiary follicle is formed from a secondary follicle when separation of granulosa cells occur to form a cavity or antrum. Concurrent with the formation of the antrum the outer border of granulosa cells is surrounded by a cell layer of stromal origin designated as the thecal layer. With enlargement and filling of the antrum with follicular fluid, the follicle grows and is designated as a mature Graafian follicle. At the onset of estrus the

follicle which will ovulate is approximately 10 mm in diameter and increases to 16 to 18 mm by ovulation. During this time, the mature follicle consists primarily of granulosa and theca cells. Generally 6 to 10 layers of granulosa cells line the antrum. This layer is avascular and is separated from the theca cells by a lamina propria. There are four to five layers of theca cells which are highly vascularized and are in opposition with the ovarian stromal tissue. The theca cells classically are divided into two layers, the theca interna and theca externa. The theca interna is composed primarily of spindle shaped connective tissue cells (fibrocytes); however, near ovulation some hypertrophy occurs and cells become epithelioid or glandular in appearance. The theca externa consists of spindle shaped connective tissue cells (Marion, Gier and Choudary, 1968; Priedkalns and Weber, 1968; Rajakoski, 1960).

Falch (1959) first introduced the concept that granulosa and theca cells may interact for biosynthesis of estrogens. Since this suggestion, much attention has focused on these two cell types to identify location and chemical biosynthetic pathways of estrogen production. Earlier work utilized in vivo and in vitro incorporation of isotopically labeled precursors to study pathways and cell types involved in the synthesis of steroids. Since the advent of the radioimmunoassay, many workers have turned their attention

to mechanisms controlling these pathways, primarily the role of gonadotropins.

The primary purpose of this section is to review three areas involved in steroid biosynthesis: pathways, cell types and controlling mechanisms.

Much of the research has been done utilizing in vitro systems and therefore is subject to criticism applicable to all in vitro systems. Pathways shown to predominate in vitro may not dominate and indeed may not be functional in the intact animal. Precursors in the medium may not be freely available in vivo. In vitro work with follicular tissue can be criticized additionally due to the nature of the tissue cells involved. Granulosa cells are avascular in vivo and are separated from the blood supply by a lamina propria. This may act as a barrier or otherwise limit availability of certain substrates to the granulosa cells. The use of follicular minces or slices would expose granulosa cells directly to all substrates in the medium. Furthermore, granulosa cells from large preovulatory follicles have been shown to undergo spontaneous luteinization in some in vitro systems, and granulosa cells from small follicles undergo luteinization following addition of LH to culture medium (Channing, 1970a, 1970b, 1974). Luteinization is the morphological and functional transformation of granulosa cells into luteal cells. Channing (1970a) stated that

granulosa cells have lutenized in culture when they accumulate eosinophillic granules and lipid droplets in the cytoplasm, increase in size and cytoplasmic-nuclear ratio, and secrete large amounts of progesterone. Histological observations on granulosa cells which luteinized in vivo revealed similar changes as well as transformation of mitochondria cristae from plate-like to tubular and villous forms, with the endoplasmic reticulum becoming agranular. These changes in organelles are associated with steroid synthesis (Priedkalns and Weber, 1968).

Several workers suggested that removal of the ovum also results in luteinization of the follicle. Surgical ovectomy in vivo resulted in luteinization and increased progesterone secretion by follicles in rabbits and pigs, whereas simple puncture of the follicle and loss of follicular fluid had no effect on follicular steroidogenesis or morphology (El-Fouly et al., 1970). In support of these findings, rat granulosa cells cultured in monolayers luteinized when cultured with limited oocytes, whereas granulosa cells cultured with many ova retained their granulosa cell morphology (Nekola and Nalbandov, 1971). These authors suggested that the ovum may produce a luteostatic substance that inhibits luteinization of the granulosa cells. This concept is not supported by the following observations.

Indomethacin blocks ovulation in the rabbit; however, luteinization occurs in response to the ovulatory surge of gonadotropins. The result is a fully functional corpus luteum with an ovum trapped within it. This does not disprove the presence of a luteostatic substance as the ovulatory surge of LH may inhibit the synthesis of such a substance by the ovum (Caldwell, Auletta and Speroff, 1973). Moor, Hay and Seamark (1975) observed that cultured sheep follicles did not luteinize following degeneration of the ova. Linder et al. (1974) reported that progesterone secretion was not increased when ova were removed from incubated rat follicles. Pig, monkey and human granulosa cells from small or medium follicles will not luteinize in culture unless stimulated by LH (Channing 1970a, 1970b, 1974).

In attempts to overcome these inherent problems of follicular tissue many workers have limited their studies to short incubations or have worked with whole or intact follicles. In this review tissue preparation and type of system utilized will be identified. Any in vitro experiment lasting less than 24 hr will be referred to as an incubation, whereas any experiment longer than 24 hr will be designated a culture.

There are two primary pathways for estrogen biosynthesis, the so called "Δ^5 and Δ^4" pathways (delta five

and delta four; Figure 1; Ryan and Smith, 1965). In the Δ^5 pathway, pregnenolone is converted to androstenedione through the Δ^5-3β-hydroxy-steroids, 17-hydroxy-pregnenolone and dehydroepiandrosterone, whereas in the Δ^4 pathway, pregnenolone is converted to androstenedione through the Δ^4-3-keto steroids, progesterone and 17-hydroxy-progesterone. Androstendione then can be converted to estradiol through either estrone or testosterone.

Workers utilizing metabolites isolated from follicular fluid (Short, 1962a) or in vivo incorporation of isotopically labelled precursors (YoungLai and Short, 1970) postulated that the Δ^4 pathway predominates in the ovaries of mares. Aakvaag (1969a) also using isotopically labelled precursors reported that androstenedione is synthesised solely via the Δ^4 pathway in porcine ovarian tissue incubated in vitro. The Δ^4 pathway also has been shown to predominate in homogenized mouse ovaries using similar incorporation techniques (Kraiem and Samuels, 1974). However, various techniques indicate the Δ^5 pathway predominates in the human ovary (Aakvaag, 1969b; Ryan and Smith, 1965; Patwordhan and Lanthier, 1971). Lacroix, Eechaute and Leusen (1974) have shown clearly that estradiol synthesis is through the Δ^5 pathway in incubated bovine follicular tissue. Isotopically labelled pregnenolone and 17-hydroxy-pregnenolone were converted by bovine follicular tissue to androgens, whereas

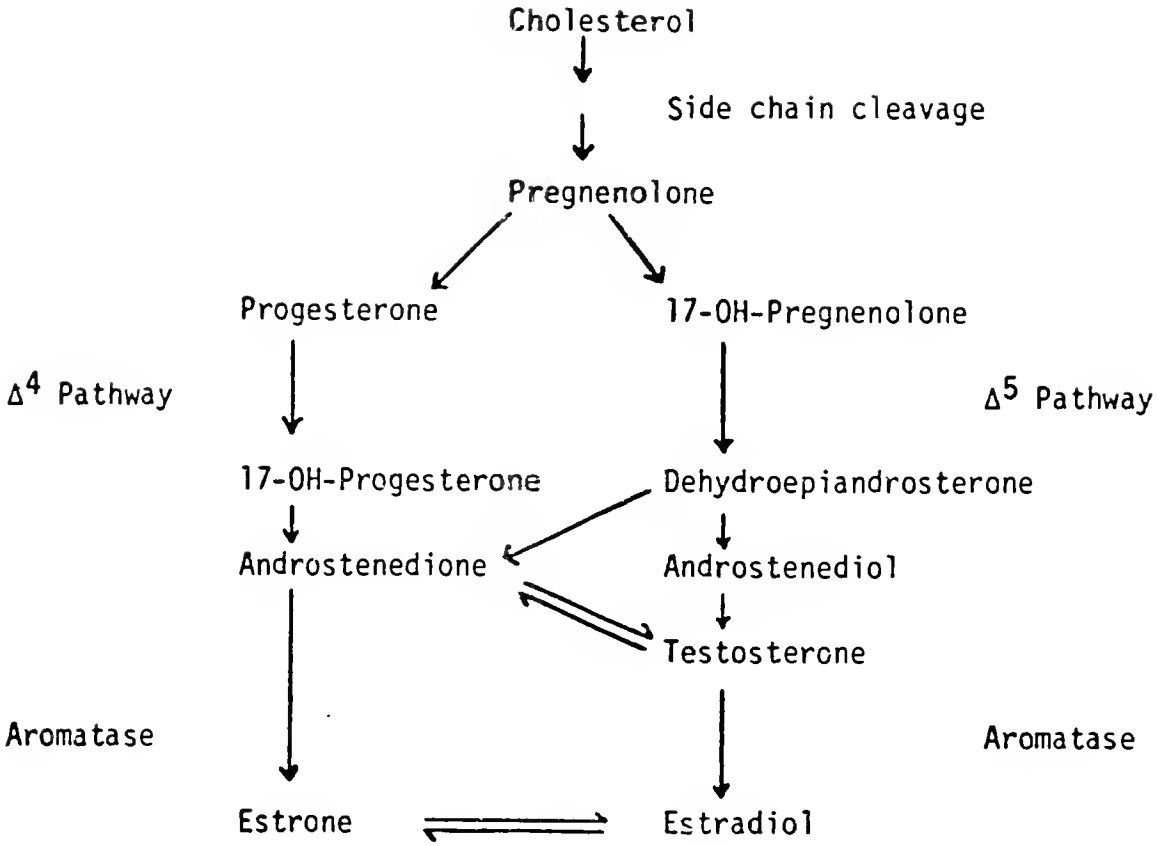

Figure 1. Estrogen biosynthetic pathways.

negligible progesterone or 17-hydroxy-progesterone were transformed.

Considerable work has been conducted to determine cell types which are responsible for the various steps within the steroidogenic pathways. Short (1962a) suggested a "two cell type" theory for ovarian steroid synthesis. This theory postulated that all steps of steroidogenesis for estrogen synthesis occur within the theca cells, and at the time of ovulation the burden of steroidogenesis is shifted to the granulosa cells which are only capable of progestin secretion. Portions of this theory have since been challenged by considerable in vitro investigations.

Human theca cells incubated alone or recombined with granulosa cells, but not granulosa cells alone, produce estradiol from ^{14}C acetate in vitro (Ryan, Petro and Kaiser, 1968). In contrast, theca but not granulosa cells from hamster follicles incubated in vitro produce high concentrations of androgens but very little estradiol. Recombination of theca and granulosa cells resulted in five times more estradiol accumulation in medium than either cell type alone (Makris and Ryan, 1975). Similar findings were reported in bovine follicular tissue incubations (Lacroix et al., 1974). Both cell types were able to convert pregnenolone to androstenedione; however, this capacity was small in the granulosa cells compared to the theca cells.

Both cell types also were able to transform androstenedione to estrogens, but granulosa cells were much more active in this regard. These studies suggest that theca cells have only limited aromatase activity in vitro. It also has been demonstrated that incubated granulosa cells from equine (Ryan and Short, 1965; YoungLai, 1972) porcine (Bjersing and Carstensen, 1967) and rabbit (YoungLai, 1973) follicles are capable of aromatization of exogenous androstenedione or testosterone to estradiol. However, equine theca cells have a higher conversion rate than do granulosa cells (Ryan and Short, 1965). From these incubation studies it appears that there is a positive interaction between these two cell types for estradiol synthesis. It has been postulated that theca cells, possibly in combination with granulosa cells, synthesize steroids up to the androgens which are then transported or simply diffuse to the granulosa cells where they are aromatized to estrogens.

A recent study by Channing and Coudert (1976) suggested that this is not the mechanism of in vivo estradiol synthesis in the rhesus monkey. In this species plasma estradiol concentrations remained unchanged for up to 120 min following surgical aspiration of granulosa cells and follicular fluid.

It is generally accepted that LH plays a major role in the regulation of gonadal steroid secretion. LH is

stimulatory to steroid secretion, and this action has been shown to be at a site common to all gonadal synthesis, the conversion of cholesterol to pregnenolone (Armstrong, 1968; Hall and Young, 1968). However this site of action has not been shown directly in follicular tissue. The role of follicle stimulating hormone (FSH) on estradiol secretion generally has been attributed to its ability to stimulate growth and development of follicles to a point where they are competent of responding to the steroidogenic action of LH (Lostroh and Johnson, 1966; Kraiem and Samuels, 1974). This concept was challenged recently by Moon, Dorrington and Armstrong (1975) and Dorrington, Moon and Armstrong (1975) who demonstrated a direct action of FSH on estradiol synthesis.

Using the immature rat and mouse as a model, it has been demonstrated that FSH and LH are both necessary for complete growth and development of the follicle. Mechanisms controlling early steps of follicle development have only begun to be elucidated. FSH stimulated growth of follicles in neonatal mice. This growth was characterized by proliferation of granulosa cells and formation of a lamina propria. However, absence of FSH diminished but did not completely prevent granulosa cell proliferation. LH was necessary for more extended growth such as secretory activity of the granulosa cells, antrum formation and maintenance of the

thecal layer (Eshkol and Lunenfeld, 1972). These data suggest that there are receptors for FSH in granulosa cells of very small follicles.

Specific LH and FSH receptors have been demonstrated in follicular tissue. Autoradrigraphic studies utilizing mature follicles from mice and rats have shown that both FSH and human chorionic gonadotropin (HCG) were specifically bound to granulosa cells whereas only HCG was bound to theca cells (Fraioli et al., 1972; Midgley, 1973; Rajaniemi and Vanha-Pertulla, 1972). HCG and LH bind to the same receptor; however, HCG is more stable under the conditions necessary for this technique and therefore has been used routinely to identify LH receptors. Apparently LH receptors are found primarily in the theca cells of sheep as well (Moor, unpublished observations cited by Weiss et al., 1976). Zeleznik et al. (1974) showed that there is a different population of follicular gonadotropin receptors in immature rats compared to the mature rat. In the immature rat FSH bound only to the granulosa cells in the same manner as the adult rat. However, HCG only bound to the theca cells in immature rats and not to the granulosa cells as in the adult rat.

The point in time or state of maturity when follicles gain the ability to synthesize steroids is unknown. However, there is ample evidence that LH and FSH both are necessary for initiation and maintenance of steroidogenesis.

Moor et al. (1973) using an organ culture system reported that the amount of estrogen secreted in vitro by follicles removed from pregnant mare serum gonadotropin (PMSg) treated sheep was higher than secretion by follicles from untreated sheep. In untreated sheep only 5% of follicles secreted high concentrations of estrogens in vitro. This was increased to 25% of follicles after a 5 min exposure to PMSg in vivo and 80% with a 24 hr exposure. This demonstrates that PMSg, which has both FSH and LH activity, stimulates estrogen synthetic pathways in sheep follicles.

Addition of LH to incubation medium caused variable increases in estradiol synthesis by follicles from estrus rabbits. However, 17-hydroxyandrogen synthesis was enhanced many times that of estradiol (Mills and Savard, 1972; YoungLai, 1974a, 1974b, 1975a). Androgen secretion also was stimulated by LH in rat follicles incubated in vitro (Lieberman et al., 1975). Estradiol secretion by quartered ovaries of immature hypophysectomized rats in organ culture was stimulated by FSH, FSH plus exogenous testosterone (an aromatizable substrate) or LH plus testosterone. LH alone had no effect on estradiol secretion and was not as effective as FSH when given in combination with testosterone (Moon et al., 1975). Estradiol secretion by cultured granulosa cells from hypophysectomized immature rats was stimulated only by addition of FSH in combination with

testosterone. FSH, testosterone, and LH alone, or LH plus testosterone, had no effect on estradiol secretion (Dorrington et al., 1975). These data provide evidence for the first time that FSH has a direct role in estradiol steroidogenesis. FSH may initiate synthesis or activation of granulosa aromatizing enzymes. These observations suggest that theca cells under the influence of LH secrete androgens which then are transported to the granulosa cells for aromatization under the influence of FSH. This may explain the action of low concentrations of LH and FSH on immature or developing follicles; however, it does not explain the effects of high concentrations of gonadotropins on large preovulatory follicles. In mature follicles high concentrations of gonadotropins have a biphasic effect on steroidogenesis. Initially all steroidogenesis, including estradiol synthesis, is stimulated by gonadotropins. This stimulation is followed by an inhibitory effect on estradiol secretion and a stimulation of progesterone secretion. This biphasic effect has been shown in several species and can be mimicked by LH alone. Mills and Savard (1973) reported that follicles removed from rabbits 2 hr postcoitum incorporated significantly more ^{14}C acetate into steroids during incubation than did follicles from unmated rabbits (estrus follicles). Incorporation by estrus follicles was stimulated by addition of LH to the incubation medium; however, LH had no significant

stimulatory effect on incorporation by follicles from mated rabbits. This clearly demonstrates the stimulatory effect of LH on follicular steroidogenesis. These data also suggested that follicles from mated rabbits may be maximally stimulated by endogenous LH and FSH released at mating and, therefore, exogenous LH had no further stimulatory effect. Follicles removed 12 hr postmating incorporated very little acetate into steroids and LH had no effect on incorporation. Therefore, in rabbit follicles, LH initially stimulates steroid synthesis, and this stimulation is followed by a rapid decline in steroidogenesis by the follicle at 12 hr after exposure to LH.

In incubated rat follicles estradiol secretion was stimulated above controls the first 6 hr of incubation after LH was added to the medium. However, estradiol secretion ceased during the next 6 hr and progesterone secretion increased (Linder et al., 1974). The inhibitory effect of LH on estradiol secretion and stimulatory effect on progesterone secretion have been shown in several experiments with sheep follicles. Estrogen secretion by whole sheep follicles can be maintained for up to 7 days in organ culture (Moor, 1973; Seamark, Moor and McIntosh, 1974). However, following the addition of LH to the culture medium, estrogen secretion declined rapidly and progesterone secretion increased over several days. Similarly follicles removed from sheep after the preovulatory

surge of gonadotropins (Seamark et al., 1974) or following infusion of LH (Moor, 1974) produced insignificant amounts of estrogens when cultured in vitro. In vivo infusion of LH, into sheep in which the corpus luteum had been removed 18 hr earlier, prevented ovaries from secreting the large amounts of estrogens which otherwise would have occurred (Moor et al., 1973).

In vivo observations in cattle also are suggestive of a biphasic effect of LH on estradiol secretion. Chenault et al. (1975) observed that peripheral plasma estradiol was elevated during the initial phase of the ovulatory surge of LH, declined 50% by 5 hr after the peak of LH, and reached very low levels several hr prior to ovulation. It is difficult to determine if this is a response to LH or FSH as Akbar et al. (1974) reported a concurrent peak of FSH at this time. However, following injection of HCG peripheral estradiol concentrations declined in cattle (Dobson, 1973).

This biphasic effect of LH may be explained very simply High concentrations of LH (endogenous or exogenous) may provide a burst of steroidogenic stimulation (presumably through the side chain cleavage enzymes) thereby increasing the general availability of precursors for estrogen synthesis. The subsequent inhibition of estradiol secretion occurs several hours after exposure to LH and most likely reflects luteinization of granulosa cells and inhibition of theca

cells. This time delay may be necessary for initiation of intracellular events associated with luteinization with one end result being termination of estradiol secretion.

Several lines of evidence support this hypothesis. Following addition of LH to cultured sheep follicles CAMP synthesis increased rapidly (Weiss et al., 1976). When mature sheep follicles were exposed to low levels of dibutyryl CAMP estrogen production declined rapidly and all steroid secretion ceased (McIntosh and Moor, 1973). When higher levels of CAMP were added, estradiol secretion was inhibited and progesterone secretion was greatly stimulated. Channing (1970a, 1970b, 1974) demonstrated that LH initiated luteinization in rhesus monkey, human, swine and equine granulosa cells cultured over several days and that this effect was mimicked by CAMP. In Channing's studies both morphological and functional luteinization occurred. These studies indicate that CAMP acts as a second messenger to cause luteinization of granulosa cells.

The progesterone increase in incubated rat follicles following the addition of LH is blocked by inhibitors of protein and RNA synthesis, whereas estradiol synthesis is stimulated. This suggests that the luteinizing effect of LH, that is the stimulation of progesterone secretion and termination of estradiol secretion, requires protein and RNA synthesis. However, the general steroidogenic effect of LH as

evidenced by the earlier stimulation of estradiol secretion is not mediated through RNA or protein synthesis (Linder et al., 1974). In immature rabbit follicles the general LH stimulatory mechanisms appear to be different. Inhibitors of protein synthesis inhibited LH induced androgen production, whereas RNA synthesis inhibitors had no effect. Thus the LH induced steroidogenesis is mediated through protein synthesis possibly by activating translation of a stable messenger RNA (YoungLai, 1975a, 1975b).

The preovulatory surge of LH does not luteinize small or immature follicles. Luteinization in response to LH may depend on presence of LH receptors in the granulosa cells. Immature rat granulosa cells had no LH receptors (Zeleznik et al., 1974), whereas mature follicle granulosa cells contained LH receptors (Midgley, 1973). Channing and Kammerman (1973, 1974) reported that granulosa cells from large, pig follicles (6 to 12 mm) bound 10 to 1,000 times more HCG than granulosa cells from small (1 to 2 mm) or medium (3 to 5 mm) follicles, demonstrating that mature follicle granulosa cells contain significantly more LH receptors than granulosa cells from small or medium follicles.

Several lines of research demonstrated that FSH stimulates LH receptors in granulosa cells. Following 2 days of pretreatment with FSH in vivo, HCG bound to granulosa cells of immature rat follicles whereas no HCG was bound to

granulosa cells of untreated immature rats (Zeleznik et al., 1974). Granulosa cells from small, porcine follicles grown in culture for 2 days with FSH bound HCG, whereas cells grown without FSH bound no HCG (Channing, 1975). In hypophysectomized immature rats treated for 1 to 4 days with estradiol, LH receptors in granulosa cells declined while rats receiving FSH instead of estradiol showed no change in LH receptors (Richards and Midgley, 1976). However, granulosa LH receptors increased markedly in rats receiving both FSH and estradiol. Furthermore, follicles in rats receiving FSH or estradiol alone became atretic following exposure to LH, whereas follicles from rats treated with FSH and estradiol responded to LH by undergoing luteinization. These data suggest that FSH and estradiol act synergistically during follicle development to induce LH receptors and thereby provide mechanisms necessary for follicles to respond to the preovulatory surge of LH.

The mechanisms controlling estradiol secretion in the bovine follicle are unknown. Only two studies, demonstrating the effects of gonadotropins on bovine steroidogenesis in vitro, have been found for this review. Results from one study were too variable for the authors to draw any conclusive statements: "These results would suggest that, in some follicles, follicle stimulating hormone in vitro may modify steroid biosynthesis" (Oakey and Stitch, 1968, p. 407). Shemesh and

Hansel (1975a) reported that LH stimulated testosterone and estradiol synthesis by bovine follicular slices.

The only other documented studies on estradiol secretion by the bovine are studies quantifying peripheral blood levels. However, most workers (Chenault et al., 1975; Dobson and Dean, 1974; Glencross et al., 1973; Henricks, Dickey and Hill, 1971; Shemesh, Ayalon and Linder, 1972; Wettemann et al., 1972) concentrated their observations during the follicular phase of the estrous cycle, with only occasional samples, if any, taken throughout the cycle. Estradiol increases for several days prior to estrus and reaches a peak on the day of estrus with the highest concentrations occurring during the preovulatory LH surge. Following the surge of LH, estradiol declines rapidly reaching very low levels several hours before ovulation. Estradiol concentrations are not as well documented during the luteal phase of the cycle. Several authors (Dobson and Dean, 1974; Glencross et al., 1973; Shemesh et al., 1972) have described high concentrations of estradiol on different days of the cycle. However, these peaks are variable from study to study, which suggests that there are no consistent increases in estradiol secretion during the luteal phase of the cycle in cattle.

Changes in blood estrogens may be correlated with ovarian follicular development. Large follicles (10 to 12 mm)

are found on the ovary at all stages of the estrous cycle, but preovulatory follicles (16 to 18 mm) are found only on days 20, 21, and 0. Follicular growth appears to be continuous and independent of stage of the cycle (Choudary et al., 1968; Rajakoski, 1960). If these large transitory follicles secrete estrogens this may explain the variable increases in blood estrogen during the luteal phase of the cycle which have been detected. With random growth, follicles would reach a certain level of maturity and begin to secrete estrogen. If the follicle has reached maturity during the proestrus period, the estrogens, in the absence of luteal progesterone concentrations, may stimulate a preovulatory surge of LH which causes the follicle to grow into a preovulatory follicle and ovulate. If the follicle reaches maturity during a period of high progesterone concentration (the luteal phase) it would secrete estrogen and then undergo atresia without inducing either a preovulatory surge of LH or ovulating.

Several authors have suggested that $PGF_{2\alpha}$ also may influence estrogen secretion in the bovine. Chenault et al. (1975) observed spikes of estradiol following IM injection of $PGF_{2\alpha}$. Hixon et al. (1973) observed elevated peripheral blood estrone and estradiol following intraluteal injection of $PGF_{2\alpha}$. These effects may be a direct effect of

$PGF_{2\alpha}$, as Shemesh and Hansel (1975a) reported that $PGF_{2\alpha}$ stimulated testosterone but not estradiol secretion by bovine follicular slices. This concept is not supported by Louis et al. (1974a) who did not observe increases in estradiol immediately following administration of $PGF_{2\alpha}$.

These observations are of interest as it has been suggested that there might be interplay between $PGF_{2\alpha}$ and estrogen for spontaneous luteolysis. It long has been known that estradiol shortens the length of the estrous cycle in intact but not hysterectomized cattle (Brunner, Donaldson and Hansel, 1969). This suggests that estradiol works through the uterus for luteolysis. It also is known that estrogens cause release of $PGF_{2\alpha}$ from the sheep uterus (Caldwell et al., 1972; Ford et al., 1975). Therefore, $PGF_{2\alpha}$ released from the uterus may stimulate estradiol synthesis in the follicle which then stimulates additional release of $PGF_{2\alpha}$ from the uterus in a reinforcing manner. However, it should be pointed out that the uterus is not necessary for luteolysis in response to exogenous $PGF_{2\alpha}$ (LaVoie et al., 1975; Stellflug et al., 1975).

There is little known about the dynamics of follicular steroidogenesis or luteolysis in cattle. These two functions may indeed be tied together and mutually stimulatory. The corpus luteum must be regressed to induce a controlled ovulation, and steroid synthesis (estrogen) is essential for

proper conditioning of the reproductive tract for reception of the egg and sperm. Considerable research is necessary before these systems will be understood fully and this knowledge is essential before these processes can be optimally controlled.

SECTION II

IN VIVO RESPONSES TO $PGF_{2\alpha}$ THAM SALT

Materials and Methods

Experiment 1. Response of Dairy Heifers to Two Injections of $PGF_{2\alpha}$ Tham Salt 12 Days Apart

This experiment was designed to determine if two injections of $PGF_{2\alpha}$ tham salt administered 12 days apart would increase both the percentage of dairy heifers in the potentially responsive phase of the estrous cycle at the second injection and the number of animals having a synchronized estrus after the second injection.

Thirty-seven cycling dairy heifers, 11 to 14 months of age, were utilized. Before being assigned to this study, all animals were examined per rectum to verify that reproductive tracts were normal and ovaries were active. All animals were treated twice, 12 days apart, with 33.5 mg $PGF_{2\alpha}$ tham salt* (IM) dissolved in 5 ml of .9% saline. Animals were observed twice daily for signs of estrous behavior from 50

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*$PGF_{2\alpha}$ tham salt used in all experiments presented in this dissertation was provided by Dr. J. W. Lauderdale, Upjohn Company, Kalamazoo, MI.

days prior to the first injection until 30 days after the second injection. Estrous detection was conducted prior to the trial to determine the exact day of the estrous cycle each animal was in at the time of the first injection.

A jugular blood sample (30 ml) was collected from each animal 1 to 3 hr prior to each $PGF_{2\alpha}$ injection. All blood samples were collected via jugular puncture into heparinized syringes, placed immediately into an ice bath, centrifuged at 12,000 g for 15 min at 4 C, and plasma stored at -20 C until analyzed for plasma progestin concentration.

Plasma progestins were measured by radioimmunoassay procedures described by Abraham et al. (1971). The antiprogesterone was a gift of Dr. J. L. Fleeger of Texas A&M University. Extraction and quantification procedures have been validated in our laboratory by Chenault et al. (1973, 1975, 1976).

Statistical analyses for differences in response to $PGF_{2\alpha}$ and progesterone concentrations were done by Chi-square and analysis of variance, respectively.

Experiment 2. Physiological Response of Dairy Cattle to a Luteolytic Dose of $PGF_{2\alpha}$ Tham Salt Administered Intramuscularly

Objectives of this study were to determine if heart rate, arterial blood pressure, uterine temperature and arterial blood temperature were affected by a luteolytic dose

of $PGF_{2\alpha}$ administered intramuscularly. Intramuscular injection is the recommended route for administration.

Thermocouple preparation and calibration

Thermocouple preparation and calibration have been described extensively by Gwazdauskas (1974) and Gwazdauskas et al. (1974). Lengths of 36 gauge, nylon coated, copper constantan wire were pulled through polyvinyl tubing (V-7; Bolab Inc., Derry, N.H.). The terminal thermojunctions were heat sealed in polyvinyl and then coated with liquid tygon (U.S. Stoneware Co.). Stranded, untinned copper extension wires were soldered to divided copper wires leading to the thermojunctions. All extension wires lead either to a millivolt potentiometer (#8686, Leads and Northrup, Philadelphia, Pa.: limits of error of recording system $\pm$ .075 C), or to a dual channel strip chart recorder (Hewlett-Packard M 7100B; limits of error of recording system $\pm$ .03 C). Most, but not all, of the potentials from the arterial ice water thermocouples were suppressed by known amounts before being amplified and recorded.

Calibration of the thermocouples was made routinely by use of a Bureau of Standards Certified Thermometer in a well stirred, insulated water bath held at intervals between 36 to 40 C.

Surgical preparation and experimental protocol

Two dairy cows with histories of normally occurring estrous cycles were used in these experiments. Prior to surgery animals were placed on a 48 hr feed and water fast. Animals were anesthetized with 2 to 4 g sodium thiopental (Abbott Laboratories, North Chicago, Ill.) dissolved in saline while standing and restrained. They then were placed on a portable surgical table, tracheotomized, and maintained under surgical anesthesia with methoxyfluorane (Pitman-Moore, Washington Cross, N.J.). After removal of hair, the abdominal and inguinal regions were scrubbed thoroughly with Betadine (Purdue Fredrick Co., Norwalk, Conn.) and rinsed with 70% alcohol.

An incision was made in the inguinal region and the external pudic artery located. A 1.5 mm diameter polyvinyl catheter (V-7; Bolab Inc., Derry, N.H.) and one thermocouple then were inserted into this artery and passed upward into the external iliac artery. The catheter was flushed and maintained patent with a heparin solution (15U per ml of .9% saline). The uterus then was exposed via a retroperitoneal approach. Using small scissors, a 3 to 4 cm tunnel was made under the serosa in the medial side of one uterine horn about 1 cm from the bifurcation. A thermocouple was inserted into this tunnel and tied in place with 000 silk thread. The

thermocouple was secured by tying the extension wires two or three times to the serosa along the uterine horn.

Thermocouple wires and catheters were tunneled under the skin within a stainless steel cannula, exteriorized through the flank, and maintained within a canvas pack which was attached to the flank with two stainless steel pins passed through a flap of skin. Thermocouple placements were confirmed prior to their surgical removal 7 to 10 days after completion of the experiments.

Experiments began 3 days after surgery to allow animals to recover from the surgical preparation. Treatments consisted of an intramuscular injection of either 5 ml of .9% saline or 33.5 mg $PGF_{2\alpha}$ tham salt dissolved in 5 ml saline. To obtain within animal comparisons of treatment effects, animals received on each experimental day a saline injection followed several hours later by a $PGF_{2\alpha}$ injection. One animal was utilized on 2 experimental days whereas the other was utilized on only 1 day to give a total of three saline and three $PGF_{2\alpha}$ injections.

Thermopotentials generated by the thermocouples were amplified and recorded continuously during each experiment. These were used to calculate external iliac blood temperature and uterine temperature. Arterial catheters were connected via miniature pressure transducers (RP-1500, Narco Bio-systems, Inc., Houston, Tx.) to a physiograph recorder

(Physiograph Desk Model DMP-4B, Narco Bio-systems, Inc., Houston, Tx.). This allowed continuous recording of arterial blood pressure and heart rate. Mean blood pressure was taken arbitrarily as diastolic pressure plus one third of the pulse pressure. For analysis and presentation of mean arterial blood pressure, uterine temperature and arterial blood temperature, recordings were averaged over 30 sec intervals for each experiment and means of all observations (n=3) for each 30 sec interval were plotted. Heart rates were counted from the continuous strip chart record of blood pressure.

Experiment 3. Physiological Response to a Luteolytic Dose of $PGF_{2\alpha}$ Tham Salt Administered Intravenously

This experiment was conducted to determine effects of a luteolytic dose of $PGF_{2\alpha}$ tham salt, infused directly into the jugular vein on heart rate, arterial blood pressure, uterine temperature and arterial blood temperature. This series of experiments was conducted with one cow which had been used in Experiment 2 (Section II). One day prior to initiation of this study, the animal was fitted with a polyvinyl catheter (V-7; Bolab) by jugular venipuncture. The catheter was filled with a heparin solution (15 U per ml of .9% saline), capped with a brass brad, and the external catheter stored in an adhesive tape pocket glued to the neck of the animal with branding cement.

Experiments consisted of infusion over a 2 min period either with saline (n=2) or 33.5 mg $PGF_{2\alpha}$ tham salt dissolved in saline (n=2) via the jugular catheter. On each experimental day (n=2) saline infusion was followed several hours later by $PGF_{2\alpha}$ infusion.

As in the previous experiment (Experiment 2, Section II), thermopotentials generated by the thermocouples were amplified and recorded continuously during each experiment. These were used to calculate external iliac blood and uterine temperatures. Arterial blood pressure and heart rate were recorded continuously via the arterial catheters. Organization of data for analysis and presentation was the same as described in Experiment 2 (Section III).

Experiment 4. Effects of $PGF_{2\alpha}$ Tham Salt on Uterine Blood Flow in Sheep

This experiment was conducted to determine the effect of an intravenous injection of a luteolytic dose of $PGF_{2\alpha}$ tham salt on uterine blood flow in sheep. Three sheep were anethetized with methoxyfluorane, the abdominal region cleaned with Betadine and rinsed with 70% alcohol, and a midventral incision made to expose the reproductive tract. Ovaries were removed and a small area of one miduterine artery isolated in the broad ligament. The adventitia was dissected from the vessel and the head of an electromagnetic

blood flow transducer (C and C Instrument, Los Angeles, Calif.) was positioned around the vessel and secured in place by suturing the transducer lead cables several times to the broad ligament. Lead cables were exteriorized through the flank area and maintained in a canvas pack sutured to the skin. A polyvinyl catheter was placed into the femoral vein, exteriorized through the flank and also maintained in the canvas pack.

The principle of operation of the electromagnetic flowmeter is based on Faraday's law of magnetic induction. The principle states that if an electrical conductor tranverses a magnetic field, a voltage will be induced across the field which is proportional to the strength of the magnetic field, length of the conductor, and the rate of transversal. Within the head of the blood flow transducer is an electrode and an electromagnet which are in contact with the outer surface of the vessel wall. In this case, a magnetic field is generated by the electromagnet and blood is the electrical conductor. When blood flows through the magnetic field a voltage is generated which is picked up by the electrode in the head of the flow transducer. The voltage from the pick-up electrode is a measurement of flow velocity, but since the cross-sectional area of the blood column is defined precisely, the device can be calibrated in terms of volumetric flow.

During experiments transducer lead cables were connected to a Narcomatic Electromagnetic Flowmeter (Model RT-500, Narco Bio-systems, Houston, Tx.). A continuous recording of blood flow (ml per min) was obtained by connecting the flowmeter to a Narco Physiograph Recorder (DMP-4B) equipped with Narco transducer couplers (model 7173) and channel amplifiers (model 7070). Blood flow was continuously recorded for a 2 hr period prior to treatment and 2 hr posttreatment. Flow transducers were calibrated in vitro as described by Roman-Ponce (1977).

Experiments were initiated several weeks after surgery. Animals had been ovariectomized to remove effects of endogenous gonadal hormones on uterine blood flow. Therefore without endogenous gonadal hormones blood flow to the uterus was very low. To increase uterine blood flow, sheep were injected intravenously with 20 µg estradiol 2 hr prior to administration of experimental treatments. Roman-Ponce et al. (1976) have shown that this dose of estradiol increases blood flow to the uterus in ovariectomized ewes. In that study, blood flow reached a peak approximately 2 hr after estradiol administration and remained elevated for a minimum of 6 hr.

Experimental treatment consisted of an intravenous injection of 8 mg $PGF_{2\alpha}$ tham salt dissolved in 5 ml saline via the femoral catheter. No control was used in this

experiment as saline infusion in previous studies utilizing these same animals had been shown to have no effect on uterine blood flow (Roman-Ponce et al., 1977).

To determine if $PGF_{2\alpha}$ has the same hypertensive effect in ewes as observed in cattle, periodic measurements of systemic blood pressure were made in two animals using a manometer.

Results and Discussion

Experiment 1. Response of Dairy Heifers to Two Injections of $PGF_{2\alpha}$ Tham Salt 12 Days Apart

It is well established that $PGF_{2\alpha}$ is not effective during the first 5 days of the estrous cycle. This lack of responsiveness limits the usefulness of $PGF_{2\alpha}$ as a practical agent for ovulation control. This experiment was conducted to determine if two injections of $PGF_{2\alpha}$ tham salt (33.5 mg; IM), 12 days apart, would increase both the percentage of animals (n=37) in the potentially responsive phase of the estrous cycle at the second injection and the number of animals having a synchronized estrus after the second injection.

Distribution of heifers among the various phases of the estrous cycle (day 0 to 5, 6 to 16, 17 to 21 and unknown) on the 2 days of injection, 12 days apart, are shown

in Figure 2. The majority of animals (6 of 8) in the unknown phase of the estrous cycle on the day of first injection were not observed in estrus during the 50 days prior to treatment. The remaining two animals were observed in estrus twice within a short period of time just prior to the first injection and therefore could not be assigned with certainty to any group. Animals in the unknown phase at the second injection had not been observed in estrus after the first injection but had triggered heat mount patches indicating they did respond to the first injection. Therefore, these heifers could not be assigned conclusively to any group.

A responsive stage of the estrous cycle and a response are when a heifer is at a stage of the estrous cycle in which $PGF_{2\alpha}$ injection will induce corpus luteum regression (responsive stage; days 6 to 21) allowing the animal to have a detected estrus (response). A nonresponsive stage would be between days 0 to 5 when a developing corpus luteum is present which will not regress following $PGF_{2\alpha}$.

Distribution of heifers differed ($P<.01$) among phases of the estrous cycle on the 2 days of injection. In a large normal population of cycling cattle 76% of animals (based on a 21 day cycle) would be expected to be in a potentially responsive stage of the cycle (not days 0 to 5) on any one day selected at random. In this study 25 of 37 heifers (68%,

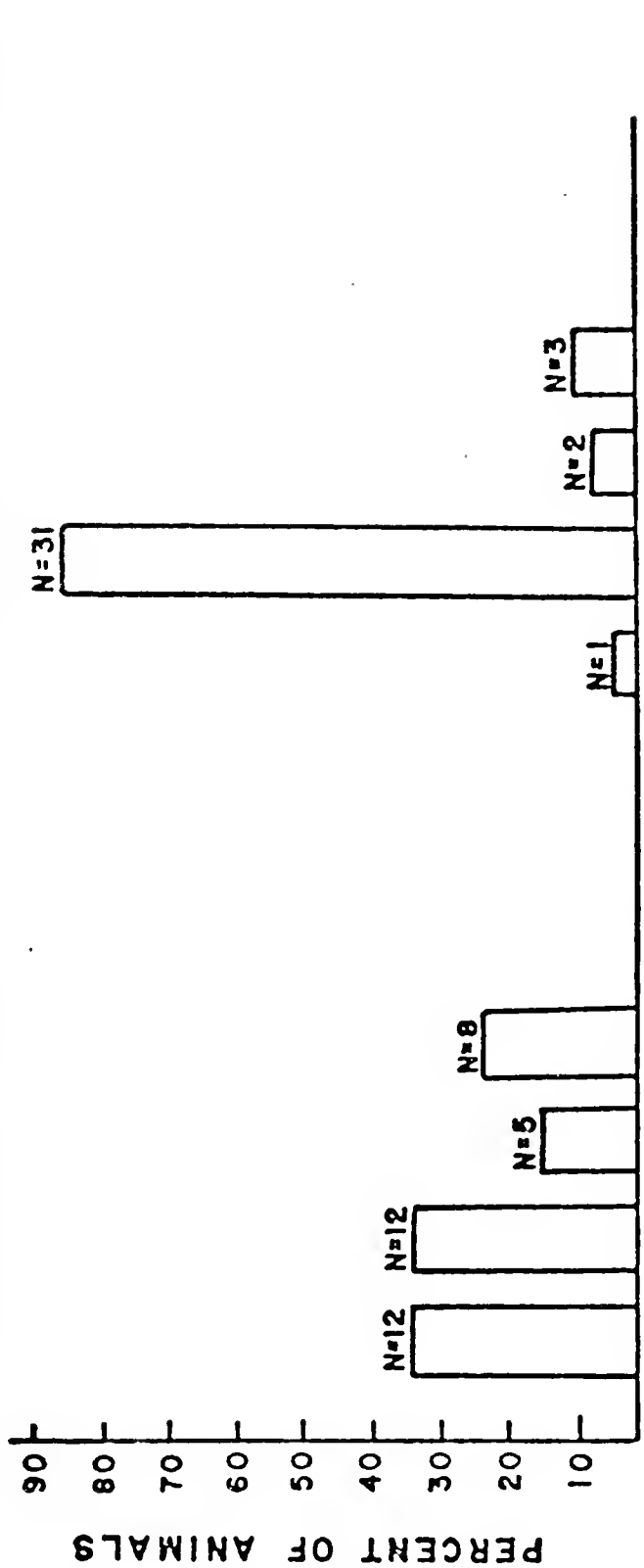


Figure 2. Heifer distribution during the estrous cycle on each injection day when two $PGF_{2\alpha}$ tham salt injections (33.5 mg) were given 12 days apart

which is very close to the expected 76%) were in a potentially responsive phase on the day of first injection, whereas 36 of 37 heifers or 97% were in this phase at the second injection day. This clearly demonstrated that a single injection of $PGF_{2\alpha}$ alters the cycle so that animals are in a potentially more responsive stage of the cycle 12 days later. This observation is substantiated by the progestin data. Average (n=37) plasma progestin concentration was higher ($P<.01$) on injection day 12 (6.51 ng per ml plasma) than on injection day 0 (3.33 ng per ml plasma). The corpus luteum is mainly responsible for plasma progestin concentrations. Thus the higher average concentration on injection day 12 indicated that more animals had a functional corpus luteum at the second injection day.

Any animal expressing estrous behavior within 8 days after $PGF_{2\alpha}$ was considered to have responded to $PGF_{2\alpha}$. The distribution of observed estrus, i.e., response to $PGF_{2\alpha}$, following both injections is shown in Table 1. Since more animals were in a potentially responsive stage of the cycle at the second day of $PGF_{2\alpha}$ injection, a greater number of animals expressed estrus within 8 days after $PGF_{2\alpha}$ compared to the estrous response of the first injection (89 versus 60% respectively; Table 1). In this study 89% of the animals responded or had a detected estrus within 8 days after the second $PGF_{2\alpha}$ injection. This demonstrated that treatment of

Table 1. Distribution of estrus in dairy heifers following injection of $PGF_{2\alpha}$ tham salt.

Day after injection	First injection		Second injection	
	Number of animals	% of animals	Number of animals	% of animals
1	4	11	1	3
2	3	7	15	40
3	10	27	4	11
4	4	11	7	19
5	0	0	0	0
6	1	3	2	5
7			2	5
8			2	5
Total				
Responding[a]	22	60	33	89**
Synchronized[b]	22	60	31	84**
Nonresponding	15	40	4	11

[a]Responding heifers were detected in estrus within 8 days after $PGF_{2\alpha}$.

[b]Synchronized heifers were detected in estrus within 7 days after $PGF_{2\alpha}$.

** $P<.01$

cattle two times, 12 days apart, was a practical management scheme to increase the percentage of animals responsive to the second injection. These results agree with Cooper (1974) who reported that 98% of heifers treated twice with 500 µg ICI 80,966 (a $PGF_{2\alpha}$ analogue) 11 days apart responded to the second injection.

If a synchronized estrus is defined as a detected estrus within 7 days after $PGF_{2\alpha}$ administration, then the percentage of heifers expressing a synchronized estrus also was greater after the second injection than after the first (84 vs 60%, respectively; Table 1). The wide distribution of estrus (8 days) following the second injection indicated that a single timed insemination may result in lowered fertility and therefore may not be practical. This indication substantiates results of Cooper et al. (1975), who reported that fertility was lower ($\tilde{=}$ 7%) following a single insemination at 72 or 78 hr after the second of two $PGF_{2\alpha}$ treatments, 10 to 12 days apart, when compared to fertility of untreated control animals or animals bred twice at 72 and 96 hr after $PGF_{2\alpha}$. Another study reported that 90% of heifers were in estrus between 48 and 72 hr after the second of two $PGF_{2\alpha}$ injections (Cooper, 1974). This degree of precision may allow for a single timed insemination with normal fertility. However, the precision of onset of estrus was not as good for our study (Table 1). Hafs et al. (1975)

reported fertility to a single insemination at 80 hr after $PGF_{2\alpha}$ was about equal to fertility of untreated controls or $PGF_{2\alpha}$ treated animals bred twice. Consequently use of a single timed insemination after the second injection still is questionable since results are not consistent among studies in the literature. Data from the present study indicated that there was considerable variability in the time of onset of estrus after the second injection and thus a single timed insemination may not be feasible using this two injection regime. A single timed insemination at 72 or 78 hr would be considerably after the time at which 43% (Table 1) of heifers were detected in estrus. These animals probably would not conceive to a single breeding at these times, however, measurement of fertility to a time insemination was not an objective of the present experiment. An alternate insemination procedure is to inseminate all animals 6 to 12 hr after onset of estrus. This would concentrate labor over a 7 to 8 day period which although is not the optimal management scheme it probably would be acceptable in commercial dairy operations.

Several systems utilizing LH releasers, either estradiol (Welch et al., 1975) or GnRH (Graves et al., 1975) have been used to synchronize time of ovulation further after $PGF_{2\alpha}$ injection. However, estradiol given at the same time as $PGF_{2\alpha}$ failed to increase fertility to a single

insemination at 72 hr after $PGF_{2\alpha}$ over fertility in animals which received $PGF_{2\alpha}$ alone and were bred at the timed insemination (Tobey and Hansel, 1975). GnRH administered at 60 hr after $PGF_{2\alpha}$ similarly had no effect on fertility rates to a timed insemination (Tobey and Hansel, 1975) whereas, when administered at 48 hr after $PGF_{2\alpha}$, fertility to a single timed insemination was suppressed (Roche, 1976b). A necessary assumption of these methods is that all animals have a mature follicle present which can ovulate in response to released LH. This may not be the case in all animals. The proestrus increase in plasma estradiol, indicative of follicular growth, is highly variable among animals following $PGF_{2\alpha}$ (Chenault et al., 1976; Louis et al., 1974a). This may explain why studies using LH releasers have failed to improve fertility to a timed insemination. The literature to date suggests that a majority of animals have a follicle which ovulates within a short period of time (99.5 ± 19.2 hr) following $PGF_{2\alpha}$ injection. Use of an LH releaser would only affect these animals and have no effect on animals which do not have a mature follicle. More research utilizing LH releasers is needed to determine if these drugs have any practical value when used in combination with $PGF_{2\alpha}$. Additional methods which induce concise follicular development in all animals may be needed before fertility to a single timed insemination can be improved to that of or above

fertility in control animals. Such methods would eliminate the human error associated with estrous detection and timing of artificial insemination. Therefore fertility rates above those presently obtained in control animals should be feasible.

Experiment 2. Physiological Response of Dairy Cattle to a Luteolytic Dose of $PGF_{2\alpha}$ Tham Salt Administered Intramuscularly

Results from Experiment 1 (Section II) indicated that $PGF_{2\alpha}$ was effective in increasing the potential percentage of animals responsive to the second of two $PGF_{2\alpha}$ injections. This experiment was designed to determine if a luteolytic dose of $PGF_{2\alpha}$ administered IM would have any detrimental effect on heart rate, arterial blood pressure, uterine temperature and arterial blood temperature in dairy cows (n=2) which may limit the practical use of $PGF_{2\alpha}$ for ovulation control.

The mean external iliac blood pressure (diastolic pressure plus one third pulse pressure) responses following IM injection of either saline or 33.5 mg $PGF_{2\alpha}$ tham salt, are illustrated in Figure 3. In response to both treatments there was a slight increase in blood pressure. Following saline injection blood pressure returned to pretreatment values by 2 min posttreatment, whereas it remained slightly

elevated ($P>.10$; <10 mm Hg) above pretreatment values following $PGF_{2\alpha}$. Associated with these initial increases were transient increases in heart rate (from 62 to 74 beats per min) following both treatments. Because saline and $PGF_{2\alpha}$ injections both caused transient increases in heart rate it was felt that the responses were due to injection and not to the drug. Apparently in response to physical stress of IM injection, catecholamines were released by the adrenal medulla which stimulated heart rate and increased arterial blood pressure. The slight elevation in blood pressure after $PGF_{2\alpha}$ injection may be due to a vasoconstricter effect of $PGF_{2\alpha}$.

Uterine and arterial temperatures were monitored as changes in these temperatures and their differences reflect changes in blood flow to certain body compartments and specifically the uterus. Dilation of peripheral vessels result in cooling of arterial blood whereas vasoconstriction of peripheral vessels results in a quick shunt of cool blood to the body core which may be followed by an elevation in blood temperature. Either decreased or increased arterial blood temperatures would indicate possible $PGF_{2\alpha}$ effects on peripheral vascular smooth muscle tone and/or alterations of thermal regulation. Alterations in uterine temperature, independent of changes in arterial blood temperatues, can be

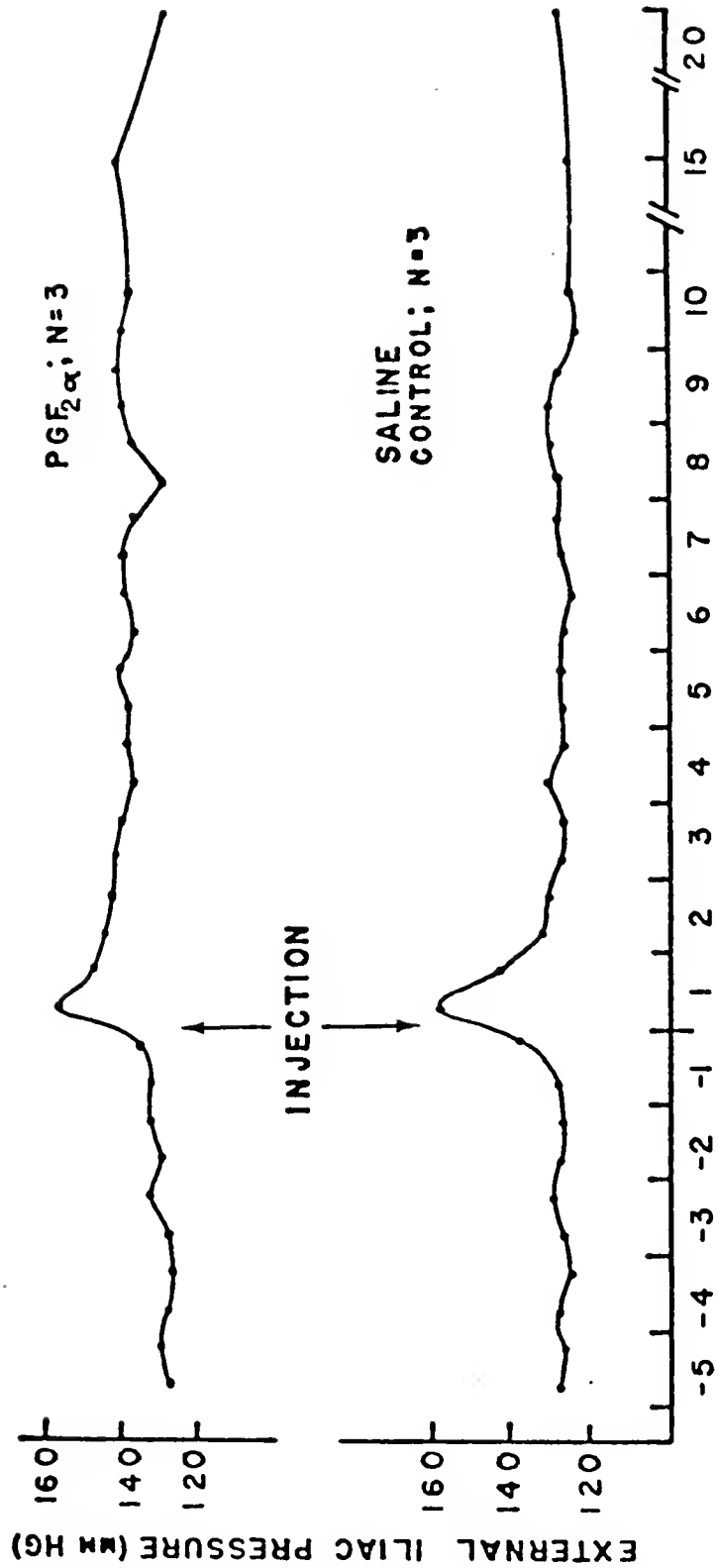


Figure 3. Mean arterial blood pressure responses to intramuscular injections of $PGF_{2\alpha}$ tham salt or saline

used as an indirect measurement of uterine blood flow. Blood is the primary means of removing metabolic heat from the uterus. Therefore if arterial blood temperature remains constant, an increase in uterine blood flow results in a decreased uterine temperature, whereas a decrease in flow results in an increased uterine temperature. In this study no changes in arterial blood or uterine temperatures could be detected for the 3 hr period following IM injection of either saline or $PGF_{2\alpha}$. Therefore, $PGF_{2\alpha}$ appeared to have no major effect on uterine blood flow. It also is likely that $PGF_{2\alpha}$ had no effect on the peripheral vascular smooth muscle or thermoregulatory mechanisms of the animals as any such effects would have resulted in some change in arterial blood temperature. Consequently a luteolytic dose of $PGF_{2\alpha}$ tham salt (33.5 mg) administered by IM injection caused no major alterations in circulatory homeostasis.

Two routes of $PGF_{2\alpha}$ administration have been used routinely in studies to date, either IM injection or intrauterine placement. When placed into the uterus lower doses, 1 to 5 mg of $PGF_{2\alpha}$ (compared to 33.5 mg for intramuscular injection) are used (Inskeep, 1973; Louis et al., 1974a; Welch et al., 1975). It is doubtful that this lower uterine dose of $PGF_{2\alpha}$ has any systemic effects, but it appears to induce a local effect since corpus luteum regression occurs. From this experiment it appears that IM

injection is a safe, practical method for administration of a luteolytic dose of $PGF_{2\alpha}$ tham salt.

Experiment 3. Physiological Response to a Luteolytic Dose of $PGF_{2\alpha}$ Tham Salt Administered Intravenously

Experiment 2 (Section II) indicated that a luteolytic dose of $PGF_{2\alpha}$ tham salt administered IM had no major effects on circulatory homeostasis or arterial blood and uterine temperatures. However, the classical smooth muscle effects of $PGF_{2\alpha}$ may have been masked by route of administration. Given intravenously, the acute availability of $PGF_{2\alpha}$ would be increased. Furthermore, giving $PGF_{2\alpha}$ as an intravenous infusion over a 2 min period also would reduce the physical stress of IM injection experienced in the previous experiment. Therefore, the intravenous approach was conducted to clarify further the effects of $PGF_{2\alpha}$ in the bovine.

Saline infusion caused no detectable effects on arterial blood temperature, heart rate, arterial blood temperature or uterine temperature. Therefore, the following discussion concerns only changes following a 2 min infusion of 33.5 mg $PGF_{2\alpha}$ tham salt.

Circulatory homeostasis (Figure 4) was affected within 70 sec from initiation of $PGF_{2\alpha}$ infusion. Mean iliac blood pressure rose rapidly from approximately 140 mm Hg to 230 mm

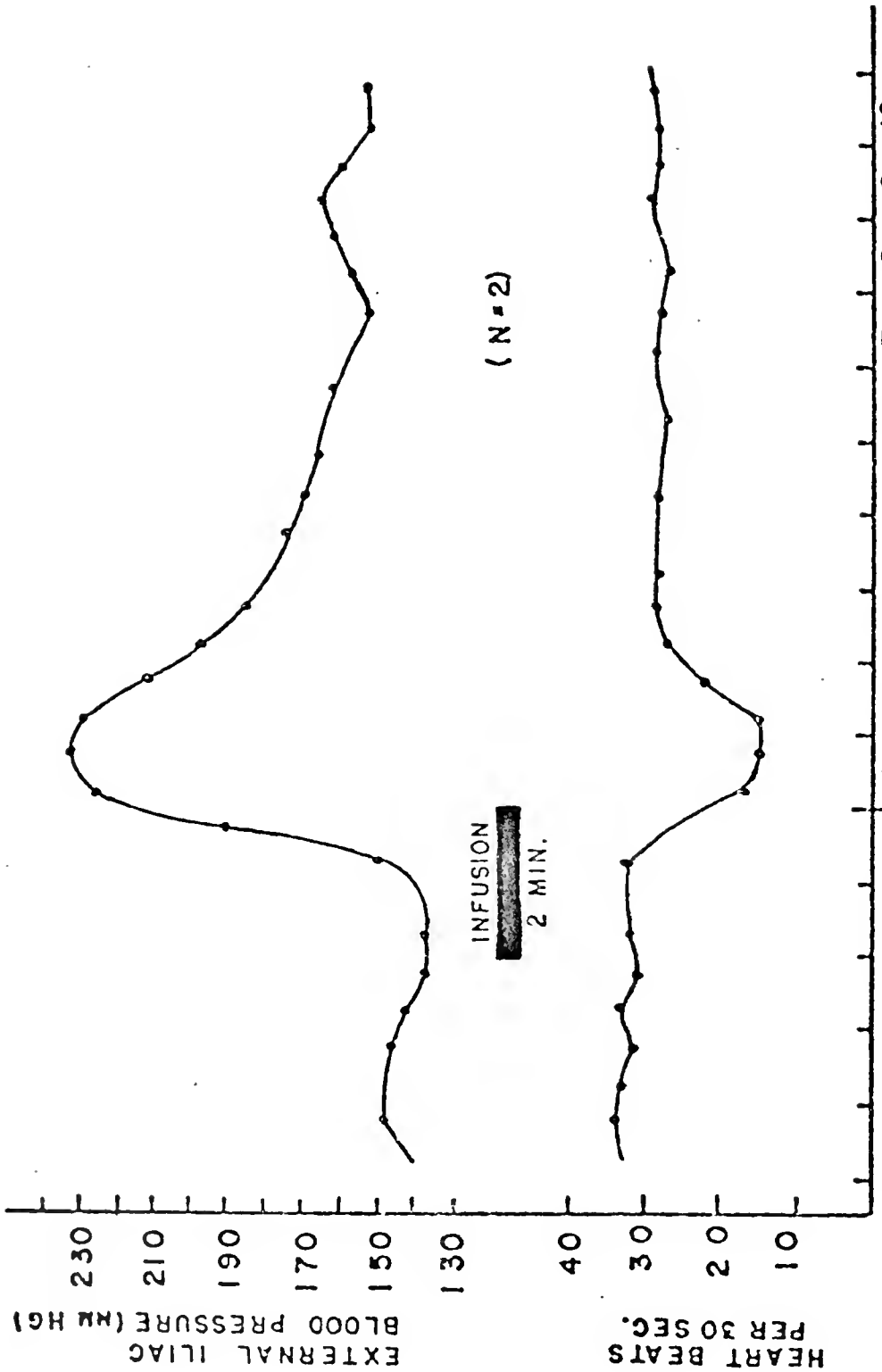


Figure 4. Mean arterial blood pressure and mean heart rate responses to two intravenous infusions of $PGF_{2\alpha}$ tham salt given within the same cow

Hg, and heart rate declined from 34 to 16 beats per 30 sec by end of the first min postinfusion. Heart rate returned to nearly pretreatment levels by 4 min posttreatment. However, blood pressure did not return to pretreatment levels until 30 min posttreatment. This increase in blood pressure agrees with that observed by Lewis and Eyre (1972) and Aitken and Sanford (1975) who reported increased blood pressure in calves following intravenous injection of the same dose (on a per kilogram basis) of $PGF_{2\alpha}$. However, both of these studies showed an increase in heart rate following $PGF_{2\alpha}$. In the present study heart rate declined and this decrease trailed the elevation in blood pressure. The decline in heart rate may be due to a physiological response to increased blood pressure and not due to a direct pharmacological effect of $PGF_{2\alpha}$ on the heart. $PGF_{2\alpha}$ has been shown to have no direct effect on the heart in several other species (Horton and Main, 1967; Lee et al., 1965; Nakano et al., 1971). No alterations in circulatory homeostasis were observed following infusion of saline.

Following IM injection of saline or $PGF_{2\alpha}$ heart rate increased (Experiment 2, Section II) which is opposite from the pharmacological response observed in the infusion experiment. This provides additional support for the conclusion that increase in heart rate following injection was a physiological response to route of administration and not to $PGF_{2\alpha}$

treatment. Apparently IM administration reduced the acute effective systemic concentration of $PGF_{2\alpha}$.

During $PGF_{2\alpha}$ infusion and for 8 to 9 min postinfusion, the cow exhibited frequent periods of defecation, urination, abdominal straining and bulging of the eyes. These observations indicated that $PGF_{2\alpha}$ probably stimulated smooth muscle activity and were consistent with the reported effects of $PGF_{2\alpha}$ on gastrointestinal smooth muscle in other species (Bennett and Fleshler, 1970; Main, 1973).

Effects of $PGF_{2\alpha}$ on uterine temperature and external iliac arterial blood temperature are shown in Figure 5. Uterine temperature increased abruptly from 39.5 to 39.8 C immediately following $PGF_{2\alpha}$ infusion. Arterial blood temperature began a slow decline 5 min after infusion and reached a stable temperature 50 min postinfusion. By 15 min postinfusion uterine temperature was also falling in parallel with the decline in arterial blood temperature.

The initial increase in uterine temperature most likely reflects an early reduced blood flow to the uterus. With a lower blood flow, less metabolic heat is removed and uterine temperature increases. A reduction in blood flow could be due either to uterine vasoconstriction or to increased transmural pressures resulting from uterine contractions. $PGF_{2\alpha}$ does have vasoconstricting effects on certain vascular beds (Viguera and Sunahara, 1969; Mark et al., 1971)

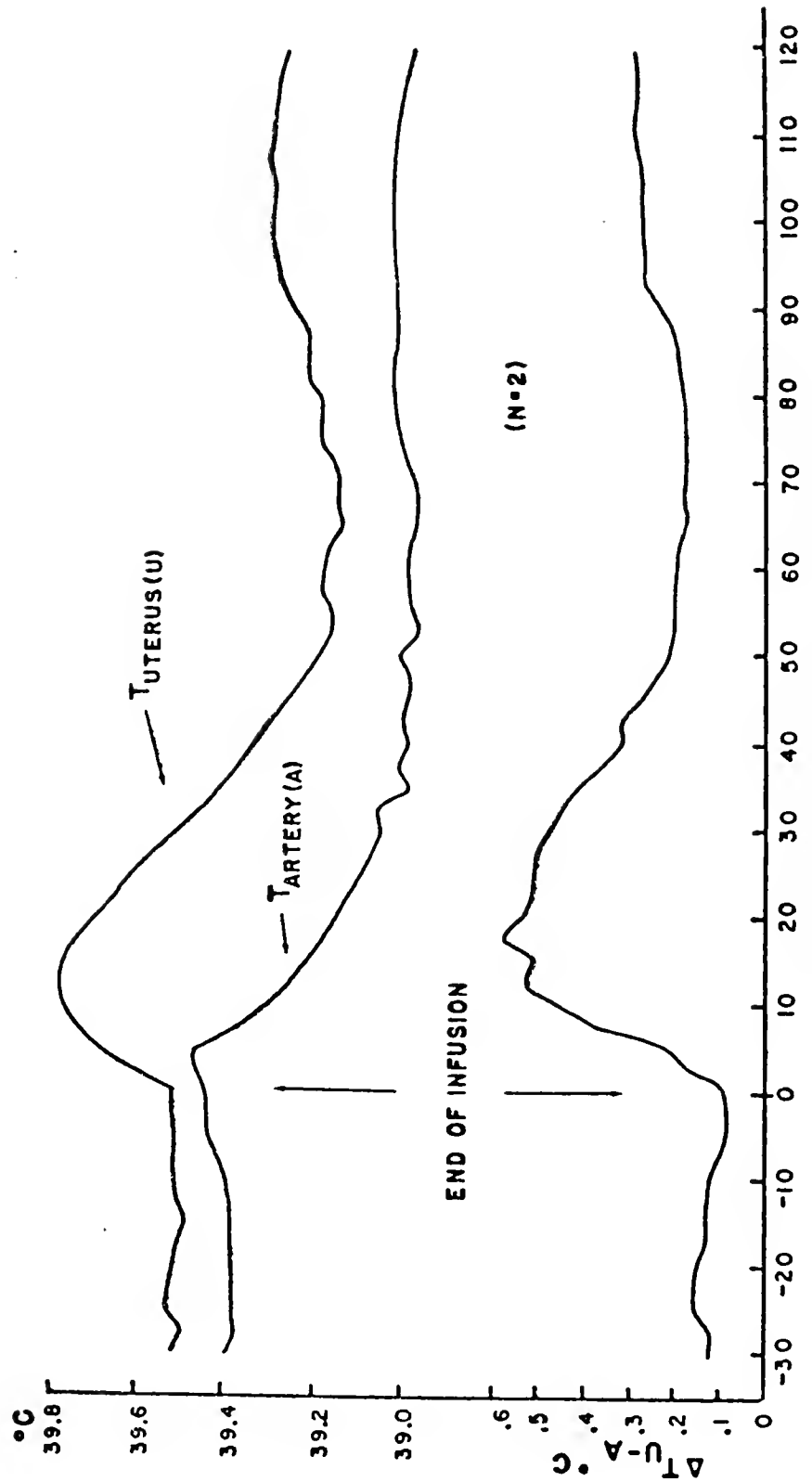


Figure 5. Mean uterine temperature and mean arterial blood temperature responses to two intravenous infusions (2 min) of $PGF_{2\alpha}$ tham salt (33.5 mg) given within the same cow

and causes uterine contractions (Capraro et al., 1976; Rexroad and Barb, 1975).

Infusion of $PGF_{2\alpha}$ appeared to affect thermoregulation as evidenced by the decline in arterial blood temperature. Splanchnic blood temperature (external iliac) decreased .4 C by 50 min postinfusion and remained stable at this temperature for the 3 hr of postinfusion recording. Saline infusion caused no change in arterial blood temperature. The mechanism by which $PGF_{2\alpha}$ may affect body temperature is unknown. However, peripheral vasodialation is a distinct possibility. Miller et al. (1976) detected a sweating response and lowered rectal temperature in mares following $PGF_{2\alpha}$ administration. In contrast, Hales et al. (1973) reported that heat generating and conserving mechanisms were stimulated by $PGF_{2\alpha}$ administered into the lateral ventricle in sheep.

Uterine and arterial temperature responses can be used to provide additional information about the physiological response of the animal to $PGF_{2\alpha}$. If arterial blood temperature changes (+ or -) and there is no change in rate of blood flow to the uterus then the temperature difference between the uterus and arterial blood (ΔT_{u-A}) would be expected not to change. However, if flood flow to the uterus is reduced, less metabolic heat would be removed and the ΔT_{u-A} would increase. Following infusion of $PGF_{2\alpha}$ a

marked widening of the ΔT_{u-A} was observed (Figure 5). This suggests that uterine blood flow was reduced markedly. The increase in ΔT_{u-A} reached a peak at 20 min postinfusion and returned to near pretreatment levels by 60 min postinfusion. This return to pretreatment levels indicated that $PGF_{2\alpha}$ had only a transient effect on uterine blood flow.

By all indications, $PGF_{2\alpha}$ had no prolonged effects on the animal, which is consistent with reports that PG's are rapidly metabolized in the body (Ferreira and Vane, 1967; Raz, 1972). The only data which contradict this are arterial blood temperatures which did not return to pretreatment levels. The rapid drop in blood temperature reflects a treatment effect (peripheral vasodilitation?). However, the stabilization at this lower temperature most likely is due simply to a loss of deep core heat. Perhaps there is also an alteration in thermoregulatory setpoint.

This study demonstrates that $PGF_{2\alpha}$ has strong smooth muscle stimulatory actions in the bovine. The animal displayed signs of discomfort during the first 10 min after infusion and the smooth muscle responses, in particular the pressor effect, may prove hazardous to animals in poor health. Therefore, the intravenous route of administration should be avoided. This route is not recommended and care should be taken when administering $PGF_{2\alpha}$ intramuscularly to avoid accidental injection into the vascular bed.

Experiment 4. Effect of $PGF_{2\alpha}$ Tham Salt on Uterine Blood Flow in Sheep

Results from Experiment 3 (Section II), using an indirect method to measure blood flow (ΔT_{u-A}), indicated that uterine blood flow in cattle was rapidly decreased by intravenous infusion of $PGF_{2\alpha}$ tham salt. This experiment was conducted to measure, by use of blood flow transducers, the direct effects of a luteolytic dose of $PGF_{2\alpha}$ (8 mg, intravenously), on blood flow through one miduterine artery in sheep (n=3).

Exogenous estradiol (20 µg) had been administered to increase uterine blood flow. At 2 hr after injection, the uterine blood flow had increased from approximately 5 ml per min to 70 ml per min. A similar response of uterine blood flow to estradiol was described by Roman-Ponce et al. (1976).

Following infusion of $PGF_{2\alpha}$ mean blood pressure (Table 2) was elevated. This response appears to be of short duration as blood pressure was returning towards pretreatment levels by 8 min postinfusion in one animal. A similar transient increase in mean blood pressure was observed in cattle in Experiment 3 (Section II). This observation indicates that in sheep and cattle responses of the circulatory system to $PGF_{2\alpha}$ are similar. This elevation in blood pressure most likely was due to a vasoconstrictor effect of $PGF_{2\alpha}$ on

Table 2. Mean arterial blood pressure (mm Hg) response of sheep to 8 mg $PGF_{2\alpha}$ tham salt administered intravenously.

Time (min) post infusion	Animal 1	Animal 2
Pre	80	70
6	134	-
8	108	96
23	-	83

vascular smooth muscle and not to an increase in heart rate, since heart rate was slowed following infusion of $PGF_{2\alpha}$ in cattle.

Uterine blood flow decreased dramatically following infusion of $PGF_{2\alpha}$ (Figure 6). Within the first min postinfusion, blood flow fell rapidly from 72 to 43 ml per min and then rebounded to 63 ml per min. Flow then declined slowly reaching its lowest flow (41 ml per min) at 10 min postinfusion. During the remainder of the experiment flow increased gradually to near pretreatment levels by 60 min postinfusion.

Immediate increases in uterine temperature and ΔT_{u-A} were observed following infusion of a luteolytic dose of $PGF_{2\alpha}$ in cattle (Experiment 3, Section II). Both of these responses are indicative of decreased blood flow to the uterus. It was concluded that $PGF_{2\alpha}$ decreased blood flow to the uterus in cattle. Results of this experiment in sheep clearly substantiate these conclusions since $PGF_{2\alpha}$ infusion caused an immediate decrease in uterine blood flow.

The initial rapid decline in uterine blood flow doubtless occurred much too rapidly for $PGF_{2\alpha}$ to be transported from the femoral vein through the heart and lungs (site of metabolism) and then to the uterus. Therefore, this response may be a neural response elicited by $PGF_{2\alpha}$. $PGF_{2\alpha}$ may stimulate the sympathetic nervous system as epinephrine has been

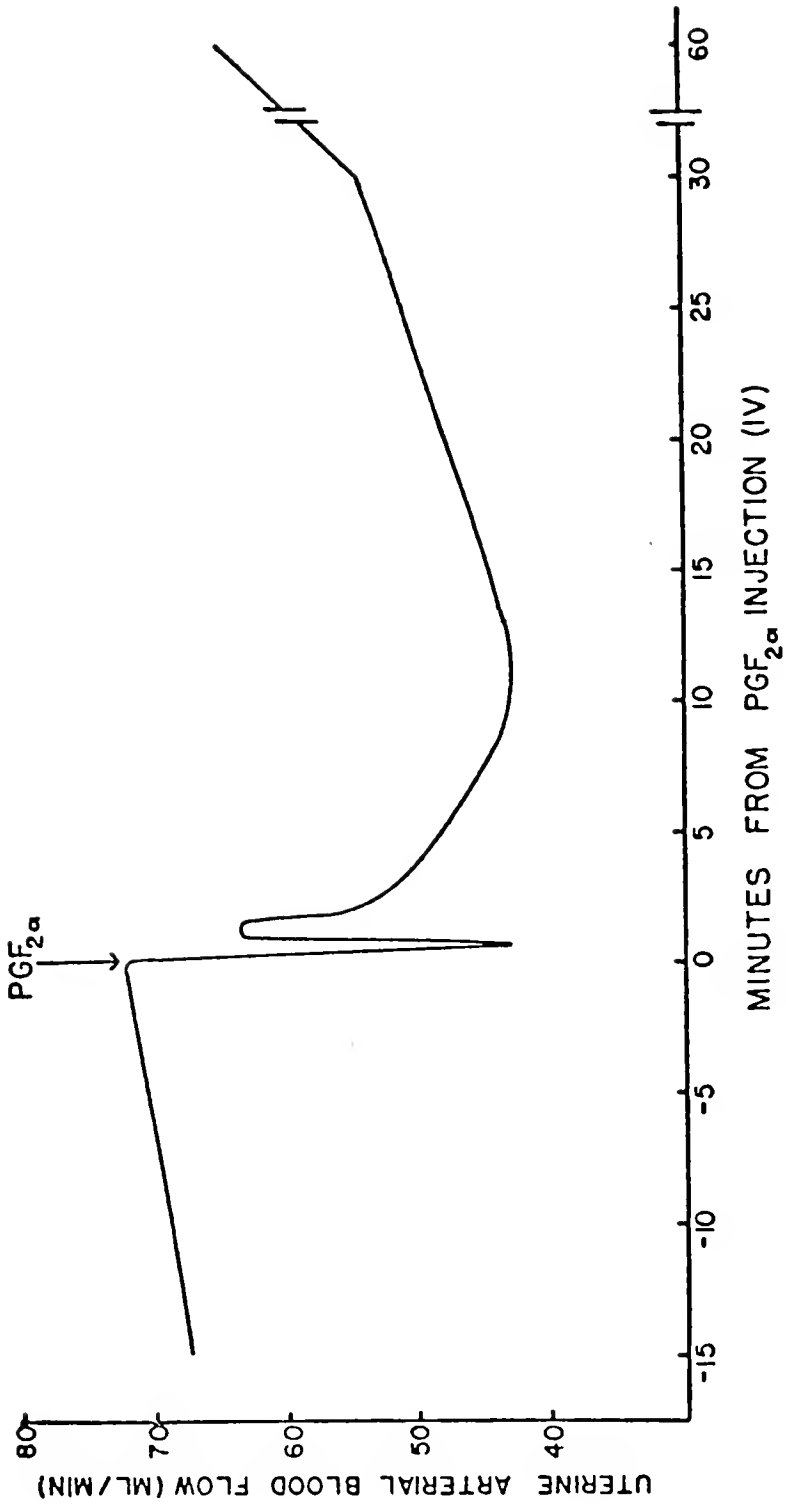


Figure 6. Uterine blood flow response to intravenous injection of $PGF_{2\alpha}$ tham salt (8 mg) in estradiol primed ovariectomized sheep (n=3)

shown to decrease uterine blood flow in sheep (Roman-Ponce, 1977) and Lauderdale et al. (1975) reported that plasma epinephrine was elevated following administration of $PGF_{2\alpha}$. This initial effect was short-lived, lasting less than 1 min, which also is suggestive of a neural effect. In contrast to this short term effect, the prolonged decline in blood flow, initiated 1 min after infusion and lasting for 60 min, most likely reflects a direct effect of $PGF_{2\alpha}$ on vascular or uterine smooth muscles. The mechanism by which $PGF_{2\alpha}$ induces this prolonged decrease in uterine blood flow is not clear from this study. In other tissues, $PGF_{2\alpha}$ has been shown to stimulate constriction of both arteries (Viguera and Sunahara, 1969) and veins (Mark et al., 1971). Similarly, $PGF_{2\alpha}$ induces uterine contractions which may result in increased transmural pressure. Any of these mechanisms could reduce blood flow to the uterus.

In ewe and cow, effects of $PGF_{2\alpha}$ were short-lived. Uterine artery blood flow returned to near pretreatment levels by 60 min posttreatment in ewes which is similar to the 50 min period required for ΔT_{u-A} to return to pretreatment levels in the cow.

SECTION III

IN VITRO BOVINE FOLLICULAR STEROIDOGENESIS

Materials and Methods

Elevated plasma estrogens have been observed in cattle following injection of $PGF_{2\alpha}$ (Chenault et al., 1976; Hixon et al., 1973) which suggest $PGF_{2\alpha}$ stimulated estrogen synthesis. Hixon et al. (1973) proposed the ovarian follicle as the site of $PGF_{2\alpha}$ stimulated estrogen synthesis. This theory is of interest as it also has been suggested that $PGF_{2\alpha}$ and estrogens interact in a reinforcing manner during spontaneous luteolysis. Furthermore, among animal plasma estrogen concentrations are highly variable following $PGF_{2\alpha}$ injection and approaching ovulation. High plasma estrogen concentrations in association with low concentrations of plasma progestins are essential for triggering the ovulatory surge of LH. Therefore $PGF_{2\alpha}$ stimulated estrogen synthesis may influence the degree of synchronization of ovulation following $PGF_{2\alpha}$ injection. In addition, there is very little known about the dynamics of follicular steriodogenesis in cattle. Objectives of these studies were to

determine the acute effects of $PGF_{2\alpha}$ and gonadotropins on bovine ovarian follicular estradiol secretion in vitro.

The in vitro approach was utilized to obtain treatment responses of isolated follicular tissue, which is not obtainable utilizing in vivo approaches. Results from these experiments should further our understanding of follicular steroidogenesis which may contribute also to our understanding of spontaneous luteolysis.

In order to obtain sufficient numbers of large follicles and faced with a limited number of cattle, animals were treated with FSH-p to induce follicular development. FSH-p (Armour-Baldwin Laboratories, Omaha, Ne.) is purified FSH obtained from pituitaries of swine and cattle. One mg FSH-p is equivalent to 1 mg of Armour Standard FSH which is a porcine FSH prepared as described by Steelman and Pohley (1953).

Lyophilized FSH-p (50 mg) was dissolved in 10 ml of .9% saline and injected intramuscularly two times daily on days 16 to 19 of the estrous cycle, with doses shown in Table 3. This treatment schedule was adopted from Bellows, Anderson and Short (1969) who used FSH-p to induce follicular development for superovulation studies.

By treating all animals in this manner it was thought that all follicles, though taken from different animals,

Table 3. FSH-p[a] injection schedule

Day of Cycle	Amount	
16	5.0 mg	2X daily
17	5.0 mg	2X daily
18	2.5 mg	2X daily
19	2.5 mg	2X daily
20	ovariectomy	

[a] FSH-p Armour Baldwin Labs.

would have developed in, and been exposed to, a similar hormonal milieu prior to harvesting. Twenty to 25 follicles of fairly uniform size (7 to 15 mm in diameter) and weight (100 to 200 mg) could be harvested per animal. Therefore a sufficient number of follicles would be obtained from one cow to run a complete experiment.

Animals were checked for estrous behavior twice daily before and during FSH-p treatment. None of the treated animals displayed estrous behavior during the injection schedule. On day 20, animals were given a local anesthetic while standing and restrained. A 10 to 15 cm incision was made in the lumbar region and ovaries removed using a cutting ecrasseur. Ovaries were placed immediately into an ice cold solution of 50% saline (.9%) and 50% incubation medium.

Incubation medium consisted of 8 parts Medium 199 with Hanks salts and glutamine (see Appendix 1; Difco Laboratories, Detroit, MI.) and 2 parts fetal calf serum (GIBCO, Grand Island, N.Y.). To each liter of incubation medium 56 mg insulin (Iletin, Eli Lilly and Co., Indianapolis, Ind.), 56 mg ascorbic acid and 18 mg gentamicin sulfate (Schering Corporation, Bloomfield, N.J.) were added. This medium has been used successfully for culture of whole sheep follicles (Moor, 1973; Moor et al., 1973) and a similar medium was used by YoungLai (1973, 1974a, 1974b) for incubation of whole rabbit follicles.

Following ovariectomy, follicles were isolated and trimmed of connective tissue, and follicular fluid was aspirated with a 22 gauge needle; follicles then were weighed and assigned randomly to treatment.

Follicles were incubated individually and free floating in 5 ml of incubation medium in 25 ml Erlenmeyer flasks; incubations were under a gaseous atmosphere of 50% N_2, 45% O_2 and 5% CO_2 in a Dubnoff shaker at 37 C for 12.5 or 14 hr. During incubation, follicles were agitated at one stroke per sec. Incubation medium was changed every 2 hr and after removal, the medium from each incubation period was stored separately at -20 C until analyzed for steroid hormones. This frequent changing of medium was conducted to allow characterization of time trends in steroid secretion during the incubation.

These studies were designed to determine acute effects of different hormonal treatments on follicular estradiol production. Several authors have utilized culture techniques to demonstrate acute treatment effects. However, in vivo, follicles grow and develop over a short period of time and then either ovulate or undergo atresia. Therefore culture systems in which follicles are maintained in a static state over long periods of time do not mimic physiological conditions. The method of preparing follicles for incubation and the incubation system used in these studies were developed

with a desire to keep the system as physiological as possible. Due to this rationale, short term incubations were used rather than culture techniques, and whole follicles were incubated rather than tissue slices or minces. Use of whole follicles maintained the integrity and interrelationship of cell layers as found in vivo which includes the physical isolation of granulosa cells from the nutrient supply. However, follicular fluid was aspirated to remove the high concentrations of steroids known to be present in follicular fluid (Short, 1962b). Otherwise, these steroids may leach out of the antrum during incubation and mask treatment effects. Moor (1973) reported that steroid secretion by whole sheep follicles in tissue culture was not affected if the follicular fluid was removed by puncture of the follicle. Similar in vivo observations have been reported in swine and rabbit follicles (El-Fouly et al., 1970).

Concentrations of estradiol, progestins and testosterone in the medium following incubations were determined by radioimmunoassays. The progestin assay was described in Materials and Methods, Experiment 1, Section II. Estradiol was measured by the technique of Hotchkiss, Atkinson and Knobil (1971). Extraction, purification and quantification have been validated in our laboratory by Chenault et al. (1973, 1975, 1976). The estrogen antibody was a gift of Dr. V. L. Estergreen of Washington State University.

Testosterone was extracted with diethyl ether, isolated by Sephadex LH-20 column chromatography, and assayed by the method of Coyotupa, Parlow and Abraham (1972). The testosterone antibody (S741 #2) was acquired from Dr. G. E. Abraham of Bal Harbor Hospital, Torrance, Cal. and has been described by Coyotupa et al. (1972).

Experimental Design

Experiment 1. Estradiol Secretion by Bovine Follicles in Vitro: Test of Incubation System

This experiment was conducted to determine if bovine follicles synthesize estradiol in this incubation system.

Ten follicles from one FSH-p treated cow were prepared for incubation as described previously and then assigned randomly to treatments. Treatment one consisted of immediate freezing of the follicles after aspiration of follicular fluid (n=5), whereas treatment two follicles (n=5) were incubated for 14 hr during which the medium was changed every 2 hr.

Follicles from treatment one were homogenized in a Polytron Homogenizer (Kinematica, distributed by Brinkmann Company, Westbury, NY.). The homogenized follicular tissue of treatment one and medium from treatment two were assayed for estradiol. Test of treatment effects on total estradiol

was conducted using analysis of variance. Least squares regression was used to determine time trends of estradiol secretion (pg estradiol per mg tissue per period of incubation, and ng estradiol per follicle per incubation period) during incubation in treatment two. The statistical model included follicle and incubation period (time) as a continuous independent variable to the highest order of significance up to the fifth order.

Experiment 2. Effects of $PGF_{2\alpha}$ and LH on In Vitro Estradiol Secretion by Bovine Follicles

This experiment was conducted to determine the effect of $PGF_{2\alpha}$ and LH on estradiol secretion by incubated follicles. Fifteen follicles from one FSH-p treated cow were assigned randomly to one of three treatments. Treatments consisted of control incubation medium (n=5), $PGF_{2\alpha}$ tham salt at a concentration of 5 ng per ml incubation medium (n=5), and LH (NIH-LH-B7) at a concentration of 50 ng per ml medium (n=5). These concentrations were chosen because they are equivalent to physiological blood levels present when dramatic changes in estrogen biosynthesis occur in vivo. $PGF_{2\alpha}$ in the uterine vein averaged under 5 ng per ml plasma on days 15 to 17 of the estrous cycle (Shemesh and Hansel, 1975b), and plasma LH often exceeds 50 ng per ml during the preovulatory surge of LH (Henricks, Dickey and Niswender, 1970; Snook, Saatman and Hansel, 1971; Thatcher and Chenault, 1976).

The incubation was for 14 hr during which the medium was changed every 2 hr. During the first 4 hr, all follicles were incubated in control medium. The first 2 hr of incubation, designated preincubation, was included to allow leaching of preexisting steroids. The second 2 hr, designated control incubation period, was included to obtain a within pretreatment measurement of steroid secretory capability. The preincubation and control periods were followed by five 2 hr treatment incubations. Hormonal treatments were added to the medium only during the treatment incubations. With each bi-hourly change of medium, fresh medium with hormonal treatments was added.

The initial design called for estradiol to be assayed in the medium from each 2 hr period. However, due to difficulty in interpreting the estradiol secretory profile, progestins and testosterone also were measured.

An extensive series of least squares analyses was conducted to determine time changes in hormonal secretion. The statistical model included treatment, follicle within treatment and time, as a continuous independent variable, to the highest order of significance. Test for significance of treatment effects was a test of heterogeneity of regression (Snedecor and Cochran, 1967). Using this test, treatment effects are significant when there is significant gain in fitting one curve for each treatment over fitting an overall pooled curve.

Experiment 3. Effects of $PGF_{2\alpha}$ on In Vivo Estradiol Secretion by Bovine Follicles

This experiment was designed to test $PGF_{2\alpha}$ dose effects on follicular estradiol secretion. Twenty follicles from one FSH-p treated cow were assigned at random to treatments containing 0, 5, 100 or 1000 ng $PGF_{2\alpha}$ tham salt per ml incubation medium. The incubation was for 14 hr including a 2 hr preincubation and six 2 hr treatment incubations. $PGF_{2\alpha}$ was added to the medium only during the treatment periods. Medium was changed every 2 hr. A control incubation period was not included in this experiment in order to begin treatments early in the incubation when steroidogenesis is maximal.

Only estradiol was assayed in the medium and statistical analyses were the same as described in Experiment 2, (Section III).

Experiment 4. Effects of FSH, Testosterone and FSH plus Testosterone on In Vitro Estradiol Secretion by Bovine Follicles

This experiment was conducted to determine if the presence of testosterone, an aromatizable substrate, FSH or testosterone plus FSH could stimulate estradiol secretion in vitro.

Twenty follicles from one FSH-p treated cow were assigned randomly to treatments in a 2 X 2 factorial design.

Treatments consisted of incubation medium alone (control), 5 X 10^{-7} molar testosterone (Steraloids, Inc., Pauling, NY.; 144.2 ng/ml) added to incubation medium, 100 ng FSH (NIH-FSH B1) per ml incubation medium, or both FSH and testosterone added to medium.

The incubation was for 12.5 hr which consisted of a .5 hr preincubation, a 2 hr control incubation, and five 2 hr treatment periods. Hormonal treatments were added to the medium during the five treatment periods (2.5 to 12.5 hr).

Estradiol was assayed in the medium from all periods and statistical analysis conducted as described in Experiment 2 (Section III).

Experiment 5. Histology of Bovine Fillicles Induced with FSH-p; Before or After In Vitro Incubation

In all preceding incubation experiments estradiol secretion declined during incubation and failed to respond to treatments. Purpose of this experiment was to examine follicles for histological evidence to account for these results.

Fifteen follicles from one FSH-p treated cow were harvested. Five follicles were incubated following the procedure described in Experiment 1 (Section III). Immediately after the 14 hr incubation these follicles were fixed in Bouins solution (Appendix 2). The additional 10 follicles

were fixed immediately in Bouins solution following their isolation from the ovary. This allowed for histological examination of follicles induced by the FSH-p injection schedule as well as follicles postincubation.

Dehydration, dealcoholization and infiltration procedures used as well as minimum time requirements of each step are given in Appendix 2. Follicles were embedded in paraffin, sectioned on a Spencer 820 Microtome (American Optical Corp., Buffalo, NY.), at 6 to 8 microns and stained with eosin and hematoxylin (see Appendix 2). Microscopic examination was conducted using a Nikon LUR-Ke microscope (Nikon Inc., Garden City, NY.).

Results and Discussion

Experiment 1. Estradiol Secretion by Bovine Follicles in Vitro: Test of Incubation System

Purpose of this experiment was to test the ability of follicles to synthesize estradiol in the incubation system. If bovine follicles secreted significantly more estradiol into the medium during a 14 hr incubation (n=5; treatment two) than was extracted from homogenized tissue of unincubated frozen follicles (n=5; treatment one) then estradiol synthesis could be assumed to have occurred during incubation.

The mean total estradiol in the medium after 14 hr of incubation, for the five incubated follicles, was 64.3 pg per mg tissue (Table 4). This was greater ($P<.01$) than the mean of 16.1 pg estradiol per mg of unincubated follicular tissue. Thus, estradiol was synthesized during incubation.

Time trends of estradiol secretion during the incubation periods were characterized by fourth order equations shown in Figure 7. These data are expressed as both pg estradiol per mg follicular tissue per period of incubation ($\hat{Y} = -5.70 + 25.135X - 10.875X^2 + 1.7913X^3 - .10300X^4$; X = hr incubation ÷ 2) and ng estradiol per follicle per period ($\hat{Y} = -1.03 + 4.241X - 1.846X^2 + .30711X^3 - .01783X^4$). As shown in this example, hormonal data expressed either adjusted for follicular weight or on a whole follicle basis resulted in nearly identical secretion curves. Furthermore, data expressed either way provided identical interpretations of treatment effects (Table 4). This was a consistent observation for all experiments and therefore hormonal results in the incubation medium will be presented only as pg hormone per mg follicular tissue.

Estradiol secretion increased initially during the first 4 hr and then declined gradually during the remainder of the incubation. Although estradiol secretion declined, there was secretion of estradiol in each period of incubation. The reason for this decline was unknown; however,

Table 4. Estradiol biosynthesis in vitro by whole follicles from FSH-p treated cows

Follicle No.	Pg per mg tissue	Ng per follicle
Frozen follicles		
1	9.5[a]	2.4[a]
2	12.6	1.7
3	16.6	3.3
4	27.3	7.2
5	14.5	2.7
Mean	16.1	3.5
Incubated follicles		
6	63.8[b]	13.5[b]
7	61.8	9.5
8	53.6	6.3
9	68.0	12.8
10	74.5	10.0
Mean	64.3**	10.4**

[a]Estradiol extracted from homogenized tissue of nonincubated frozen follcles.

[b]Total estradiol secreted during 14 hr incubation with medium changed every 2 hr.

**$P < .01$ Significantly more estradiol in medium after 14 hr incubation than extracted from nonincubated, frozen follicles.

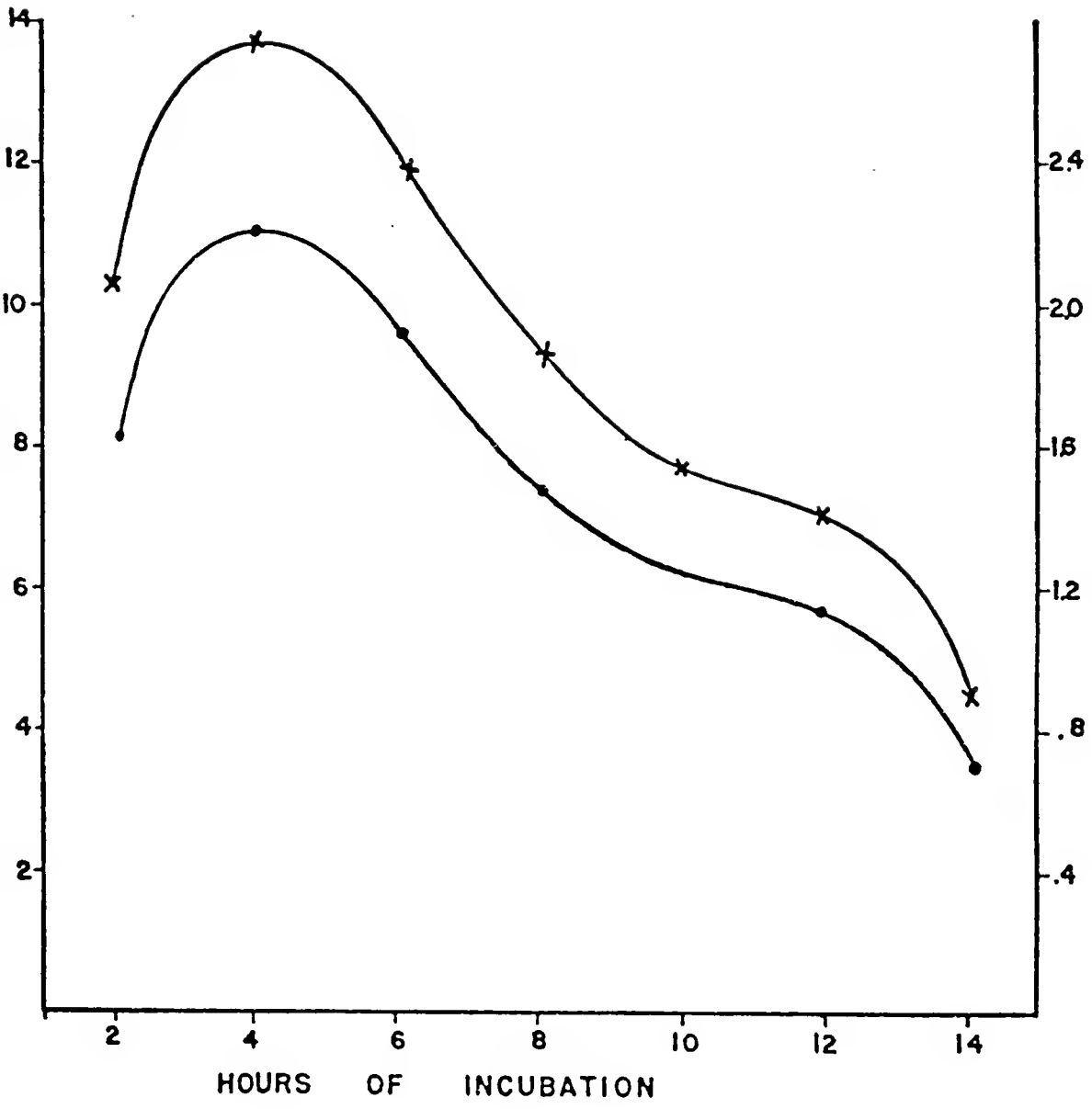


Figure 7. Estradiol secretion by FSH-p induced bovine follicles in vitro

similar results were observed in a previous experiment utilizing follicle halves incubated in this system (Chenault et al., 1975). In that study follicles were obtained from animals at various stages of the estrous cycle. One half of five follicles was frozen and the other half incubated for 24 hr during which the medium was changed every 3 hr. An additional five follicles were cut in half and each half individually incubated. There was significant heterogeneity of variance between concentrations of estradiol secreted into the incubation medium and concentrations in homogenized tissue of unincubated follicle halves. Statistical analysis conducted on a log transformation of the data indicated that significantly more ($P<.01$) estradiol was released into the medium over a 24 hr incubation than was extracted from frozen follicle halves (Table 5). This provides further evidence that follicular tissue synthesizes estradiol in this incubation system. However, estradiol secretion declined throughout the incubation periods.

Total estradiol secreted by the halves within a follicle was not different (Table 5); however, estradiol secretion patterns over time for halves within a follicle were not parallel ($P<.01$). These results suggest that hormonal secretion by half of one follicle cannot be used as a within follicle control for tests of treatment effects. For this reason, follicle halves were not used in subsequent

Table 5. Total estradiol (pg per mg per 24 hr) in half follicles

Follicle	Follicle half: Incubated[a]	Follicle half: Frozen[b]
1	5876[c]	593
2	2308	649
3	295	36
4	1367	81
5	83	49
Average	1985.8	281.6
	Incubated	Incubated
6	8600[d]	6293
7	344	535
8	1416	1449
9	3688	3452
10	1027	160
Average	2696.4[e]	

[a]Estradiol secreted during 24 hr incubation.

[b]Estradiol extracted from homogenized tissue of frozen half.

[c]Significant difference ($P<.01$) in estradiol concentration between incubated and frozen halves.

[d]Significant differences ($P<.01$) among follicles as represented by estradiol secretion of follicle halves.

[e]No differences in total estradiol secreted over 24 hr among replicates within follicles.

experiments. Great differences in estradiol secretion between follicles, as represented by half follicle secretions, also were observed ($P < .01$). This large variability between follicles harvested on various days of the estrous cycle prompted use of FSH-p to obtain a more homogeneous crop of follicles from one animal for subsequent studies.

Results of these incubations demonstrate that bovine follicles secrete estradiol in vitro in this incubation system and that estradiol secretion declined throughout all periods. This system appeared adequate for determination of acute effects of hormonal treatments on follicular estradiol secretion in vitro. Estradiol secretion by untreated control follicles should decline during incubation, whereas estradiol secretion in follicles administered hormonal treatments which may stimulate estradiol secretion should either increase, be maintained at a constant level, or decrease at a slower rate than controls. Consequently, estradiol secretion time trends of follicles within treatment groups which stimulate secretion will not be parallel to time trends of estradiol secretion of control follicles. This lack of parallelism can be tested statistically and used as a test of treatment effects.

Experiment 2. Effects of $PGF_{2\alpha}$ and LH on In Vitro Estradiol Secretion by Bovine Follicles

Results from Experiment 1 (Section III) demonstrated that follicles synthesized estradiol in vitro with the described incubation system. Purpose of this experiment was to determine effects of a physiological concentration of either $PGF_{2\alpha}$ (5 ng per ml medium) or LH (50 ng per ml medium) on in vitro follicular estradiol secretion. Following a 2 hr preincubation and a 2 hr control incubation, follicles were incubated with hormonal treatments for 10 hr.

There was no detectable heterogeneity of regression between the individual treatment estradiol secretion curves (Figure 8). The pooled estradiol secretion curve was described by a second order equation [$\hat{Y}$ (pg estradiol per mg tissue) = $186.46 - 34.3059X + 1.9732X^2$; X = hr of incubation ÷ 2]. Therefore, as compared to controls neither $PGF_{2\alpha}$ nor LH affected follicular estradiol secretion in vitro. For purposes of data presentation, each treatment group estradiol secretion curve is presented (Figure 8).

Shemesh and Hansel (1975a) reported that $PGF_{2\alpha}$ failed to stimulate bovine follicular estradiol secretion in vitro which agrees with this study. However, in that study, LH stimulated estradiol secretion which is not in agreement with data from the present study. This discrepancy may be

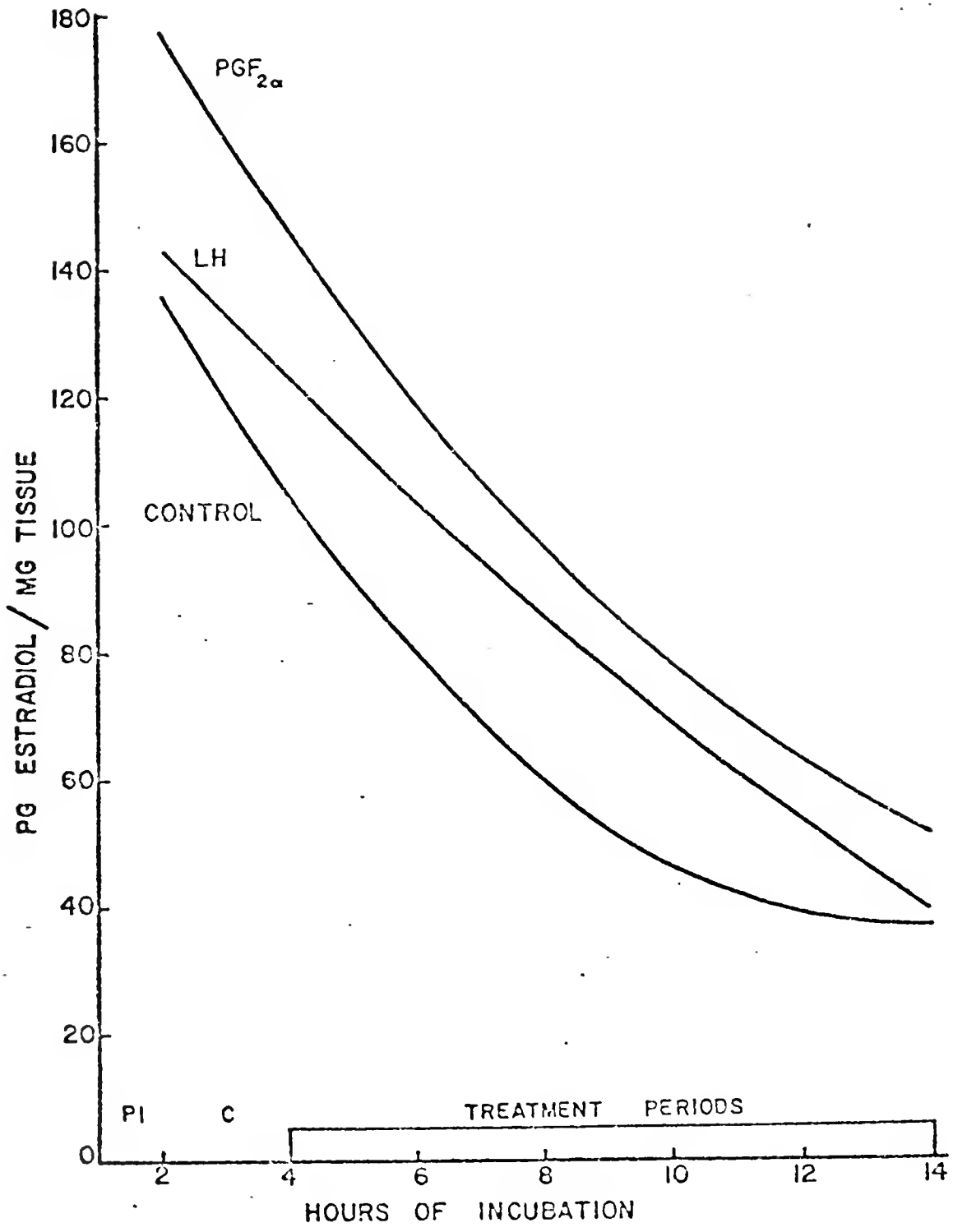


Figure 8. Effects of $PGF_{2\alpha}$ and LH on in vitro estradiol secretion by bovine follicles

due to differences either in the incubation systems or the concentrations of LH used in the two studies. Shemesh and Hansel incubated follicular slices with 5 µg LH per ml medium which is 100 times the concentration used in this study. It is also 100 times higher than the highest physiological level found in bovine plasma. In the present study, 50 ng LH per ml medium was used which is a physiological level found at the peak of the preovulatory LH surge.

Despite the fact that follicles were assigned at random to treatment groups, follicles in the $PGF_{2\alpha}$ group were more steroidogenic during pretreatment periods than follicles in the other two treatments (Figure 8). This emphasizes the need for a control incubation. If the control period had not been included, then a higher estradiol secretion rate may have been attributed to $PGF_{2\alpha}$ and not to the greater intrinsic steroidogenic capability of follicles assigned at random to this treatment group.

Follicle estradiol secretion declined within all treatments during incubation. Both LH and $PGF_{2\alpha}$ failed to alter the within follicle estradiol secretion profiles. Several alternatives may account for these results. Follicular luteinization during incubation may be one reason for the decrease in estradiol secretion. Aspiration of follicular fluid could have removed the ovum. Such a loss may initiate luteinization of the follicle as reported by El-Fouly et al.

(1970) and Nekola and Nalbandov (1971). Channing (1970a, 1970b, 1974) reported that granulosa cells from large follicles of monkeys, swine, horses or humans undergo spontaneous luteinization when grown for several days in culture and that LH induces luteinization in small follicles. Luteinization is associated with inhibition of estradiol secretion and stimulation of progesterone secretion (Moor et al., 1973; Seamark et al., 1974). However, in the present study, there was no evidence that the decline in estradiol secretion rate, indicative of luteinization, was greater for the LH treatment group. Progestin analyses were conducted to characterize progestin secretion profiles during the incubation periods in an attempt to clarify possible changes in follicular steroidogenesis due to luteinization.

There was significant heterogeneity of regression ($P<.01$) for the progestin secretion curves (Figure 9). This indicated that the progestin secretion profiles among the three treatments were not the same, i.e., that there were treatment effects. Progestin secretion in the control follicles increased throughout the incubation period and was described by a fourth order regression equation ($\hat{Y} = 58.84 - 13.580X + 8.1987X^2 - 1.2931X^3 + .07813X^4$; $R^2 = .828$). This overall continuous increase in progestin secretion is evidence that follicle cells may be luteinizing and that cellular components of the follicle are viable throughout incubation.

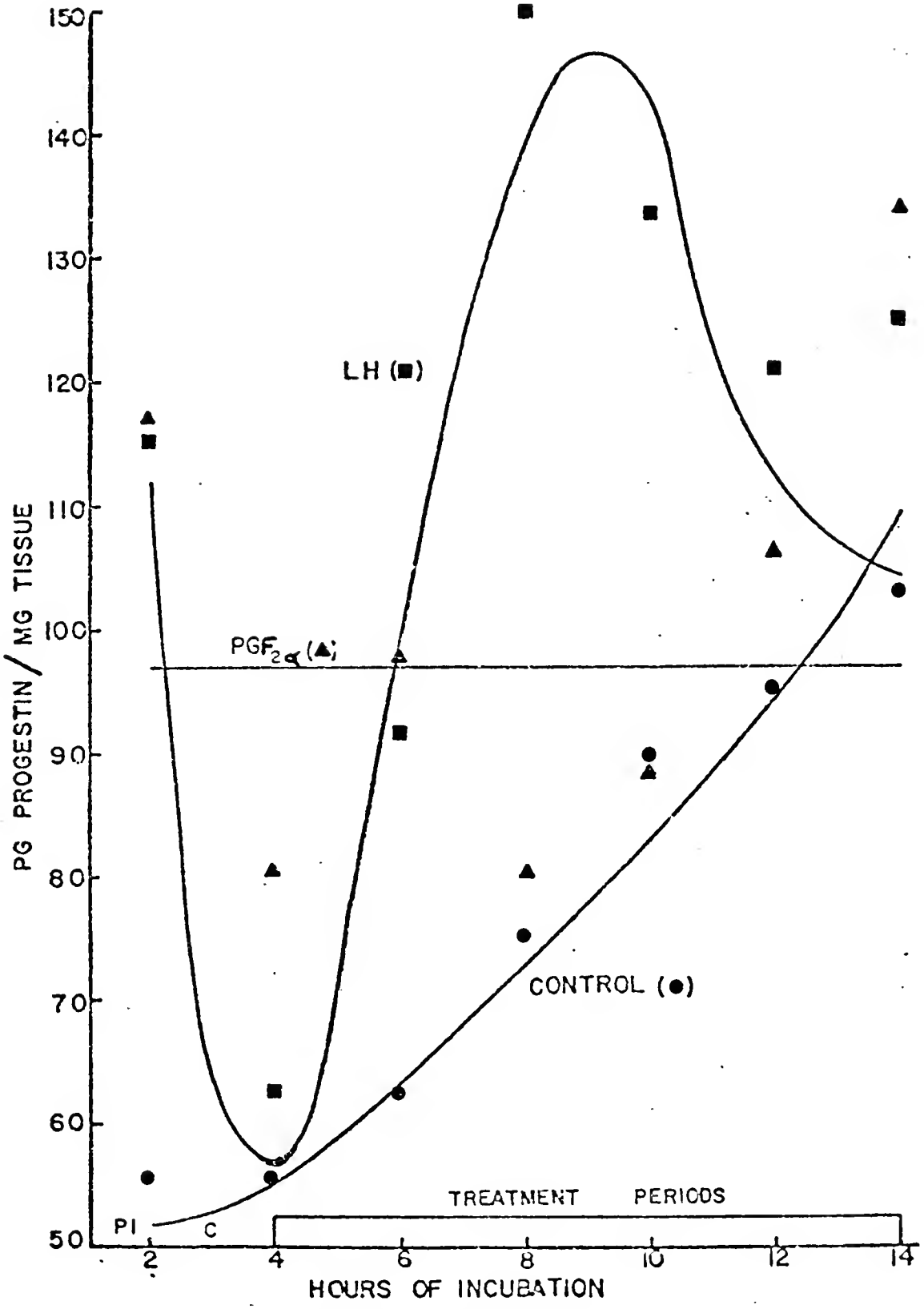


Figure 9. Effects of $PGF_{2\alpha}$ and LH on in vitro progestin secretion by bovine follicles.

The progestin response to LH is of interest since LH will induce luteinization (Channing, 1970a, 1970b, 1974). LH stimulated progestin secretion for approximately 5 hr of treatment and then declined during the latter periods of incubation (10 to 14 hr of incubation) compared to the profile of the control group. The overall fourth order regression equation for the LH treatment group ($\hat{Y}$ = 406.21 -473.411X + 213.7883X^2 -36.3689X^3 + 2.08711X^4) accounted for 65% of the variation (R^2 = .647). The lower R^2 is reflected by the fact that the actual LH treatment means (■ , Figure 9) at 12 and 14 hr were higher than the predicted values from the regression equation. Nevertheless, the progestin response indicates that LH on the average increased progestin secretion. This may be due to the general stimulatory effect of LH on steroidogenesis or luteinization during the treatment period.

No significant time trend was detected for the follicular progestin response to $PGF_{2\alpha}$. The line labelled $PGF_{2\alpha}$ (Figure 9) is the overall least squares mean of progestin secretion (96.7 pg per mg tissue per 2 hr incubation) for this treatment group. The lack of a systematic time trend indicated that a progestin response to $PGF_{2\alpha}$ was highly variable and that perhaps $PGF_{2\alpha}$ treatment delayed luteinization. Mean progestin secretion for LH and $PGF_{2\alpha}$ groups declined dramatically from the preincubation to the control

incubation periods before addition of either LH or $PGF_{2\alpha}$. This drop may be due to leaching of a high concentration of preexisting progestins from the follicles. Estradiol concentrations also declined during these pretreatment periods. From the control incubation period (2 to 4 hr), throughout the end of incubation, mean progestin secretion (▲; Figure 9) by the $PGF_{2\alpha}$ treated follicles paralleled progestin secretion in the control follicles.

Analysis of progestin production among treatments indicated that steroidogenic luteinization occurred during incubation, that LH enhanced the progestin secretion rate, and that cells were viable at the end of incubation since secretion within control and $PGF_{2\alpha}$ follicles was greatest at that time.

An alternate hypothesis to explain the decline in estradiol secretion with time is that specific enzyme systems in the estradiol biosynthetic pathway may be inhibited or their production decreased during incubation. If this is the case, steroid substrates for these enzymes would accumulate in the incubation medium. Seamark et al. (1974) reported that during organ culture of sheep follicles estrogen secretion gradually declined and testosterone secretion increased. This was followed by a decline in testosterone and an increase in 17α- hydroxylated progestins. These workers suggested that the decline in estrogens reflected a gradual

loss of the aromatase enzyme activity in the follicles which resulted in the build up of testosterone the substrate for this enzyme. The subsequent increase in hydroxprogestins would be a result of loss of enzymes in the steroidogenic pathway between pregnenolone and testosterone. The increased progestin accumulation during the incubation, as described in the present study, can be explained by such a loss of enzymes. In the bovine follicle estrogens are produced through the Δ^5 pathway (Lacroix et al., 1974). If enzymes are lost in this pathway, steroid precursors may be shifted into the Δ^4 pathway with a resultant increase in progestin secretion.

To determine if the decline in estradiol secretion was due to a loss of the aromatase enzyme activity, testosterone in the medium was measured. Testosterone was chosen because it is an immediate precursor for the aromatase enzyme and should be found in increasing concentrations during the incubation if the activity of this enzyme is being lost during incubation.

Despite randomized assignment of follicles to treatments, testosterone secretion was considerably higher during preincubation and control incubation periods for the $PGF_{2\alpha}$ group (Figure 10). This high rate of testosterone secretion is not a $PGF_{2\alpha}$ effect because it occurred during the pretreatment periods. A higher steroidogenic capability of

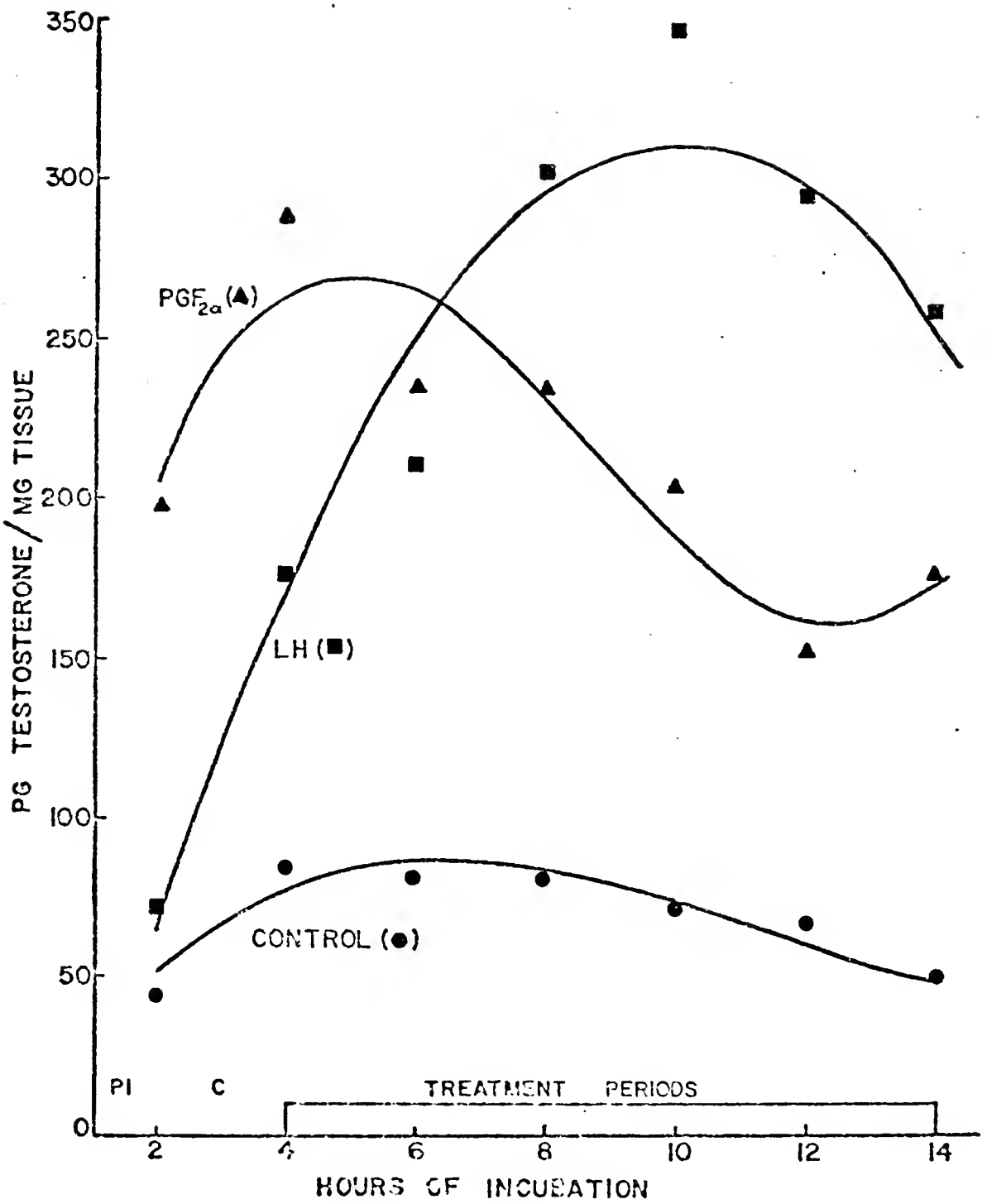


Figure 10. Effects of $PGF_{2\alpha}$ and LH on in vitro testosterone secretion by bovine follicles (PI = preincubation periods; C = control period)

follicles in the $PGF_{2\alpha}$ group also was evident when describing the estradiol response (Figure 8). The high testosterone secretion of these follicles could have been expected as testosterone is an immediate precursor of estradiol. There was significant heterogeneity of regression among treatments for the testosterone secretion curves. Testosterone secretion increased in all follicle groups between the preincubation and control incubation periods (Figure 10). This increase occurred when estradiol secretion was declining which suggests that activity of the aromatase enzyme may be lost very early in the incubation. Following this initial increase, testosterone secretion declined in a similar manner in the control ($\hat{Y} = 5.21 + 58.547X - 12.8441X^2 + .77057X^3$) and $PGF_{2\alpha}$ ($\hat{Y} = 65.52 + 190.841X - 53.7779X^2 + 4.10728X^3$) treatment groups during the treatment incubations. This decline in testosterone secretion may be due to loss of additional enzyme activities earlier in the biosynthetic pathway (pre-testosterone) as suggested by Seamark et al. (1974).

The addition of $PGF_{2\alpha}$ to the incubation medium appeared to have no effect on bovine follicular testosterone secretion in this incubation system. This is not in agreement with Shemesh and Hansel (1975a) who reported that 5 µg $PGF_{2\alpha}$ per ml medium added to slices of bovine follicles stimulated testosterone secretion. This concentration of $PGF_{2\alpha}$ (5 µg) is considerably higher than the 5 ng per ml concentration

used in the present study. A higher dose range of $PGF_{2\alpha}$ may be warranted as concentrations of $PGF_{2\alpha}$ in follicular fluid are considerably higher than blood levels (personal observation). The discrepancy between studies may be explained by this difference in concentration of $PGF_{2\alpha}$, or due to differences in tissue preparation. Shemesh and Hansel incubated follicular slices from ovaries obtained at days 16 or 17 of the estrous cycle, whereas in this study, whole follicles harvested from FSH-p treated cows were utilized.

In contrast to the decline in testosterone secretion during the treatment incubations in the control and $PGF_{2\alpha}$ follicles, LH markedly stimulated testosterone secretion throughout the treatment incubations (Figure 10; $\hat{Y} = -73.86 + 153.077X - 15.1996X^2$). This stimulation paralleled the increase in progestin secretion (Figure 9) in the LH treated follicles. LH stimulated progestin and testosterone secretion during the first 2 hr of addition to the medium and secretion was maximal by 4 to 6 hr after addition. This rapid LH effect on steroidogenesis likely was due to the general stimulatory action of LH which has been described in several species (Linder et al., 1974; Mills and Savard, 1972; YoungLai, 1974a, 1974b). Shemesh and Hansel (1975a) also reported that LH stimulated bovine follicular testosterone secretion. These observations identify the bovine as another species in which LH has a general stimulatory affect on follicular steroidogenesis.

Results from this treatment futher suggest that aromatase activity is being lost or inhibited during incubation. High concentrations of testosterone, an aromatizable substrate, (induced by LH in the medium), were present when estradiol secretion was declining. Several reports (Mills and Savard, 1972; YoungLai, 1974a, 1974b) have noted that following addition of LH to incubation medium androgen secretion was stimulated proportionately more than was estrogen secretion. In several species, including the bovine, theca cells have limited aromatization capability in vitro (Lacroix et al., 1974; Markris and Ryan, 1975), whereas granulosa cells of most species were capable of in vitro aromatization of androgens to estrogens (Ryan and Short, 1965; Bjersing and Carstensen, 1967; YoungLai, 1973). Dorrington et al. (1975) reported that granulosa cells from hypophysectomized immature rats converted testosterone to estradiol only when FSH was present in the medium. These reports suggest that LH stimulates theca cell androgen synthesis without directly affecting estradiol synthesis, and that granulosa cells under the influence of FSH convert these androgens to estrogens. Results from this study concur with this concept. Follicles in this study had been exposed to endogenous and exogenous FSH and estradiol was being synthesized. This estradiol secretion was unaffected by LH whereas testosterone secretion was stimulated.

Furthermore estradiol secretion declined in the LH treatment incubation when testosterone secretion was high. This observation could be explained in either of the following ways.

FSH may be limiting and therefore the stimulus for aromatization would be deficient, or there may be a physical disruption of the hypothesized bicellular estrogen synthetic pathway which would prevent testosterone from reaching the granulosa cells. If either of these are occurring during incubation, then estradiol synthesis would decline and testosterone would accumulate in the medium as observed.

Experiment 3. Effect of $PGF_{2\alpha}$ on In Vitro Estradiol Secretion by Bovine Follicles

In Experiment 2 (Section III) a low concentration of $PGF_{2\alpha}$ (5 ng per ml medium) had no effect on in vitro estradiol secretion. This experiment was designed to investigate $PGF_{2\alpha}$ dose effects (0, 5, 100 or 1000 ng per ml medium) on bovine follicular estradiol secretion.

Within follicle, estradiol secretion curves are graphed in Figure 11. There was no evidence that the estradiol secretion curves among treatments were not parallel. The overall pooled estradiol secretory profile was best described with a second order equation ($\hat{Y} = 185.95 - 30.439X + 1.5844X^2$. These results agree with those of Shemesh and

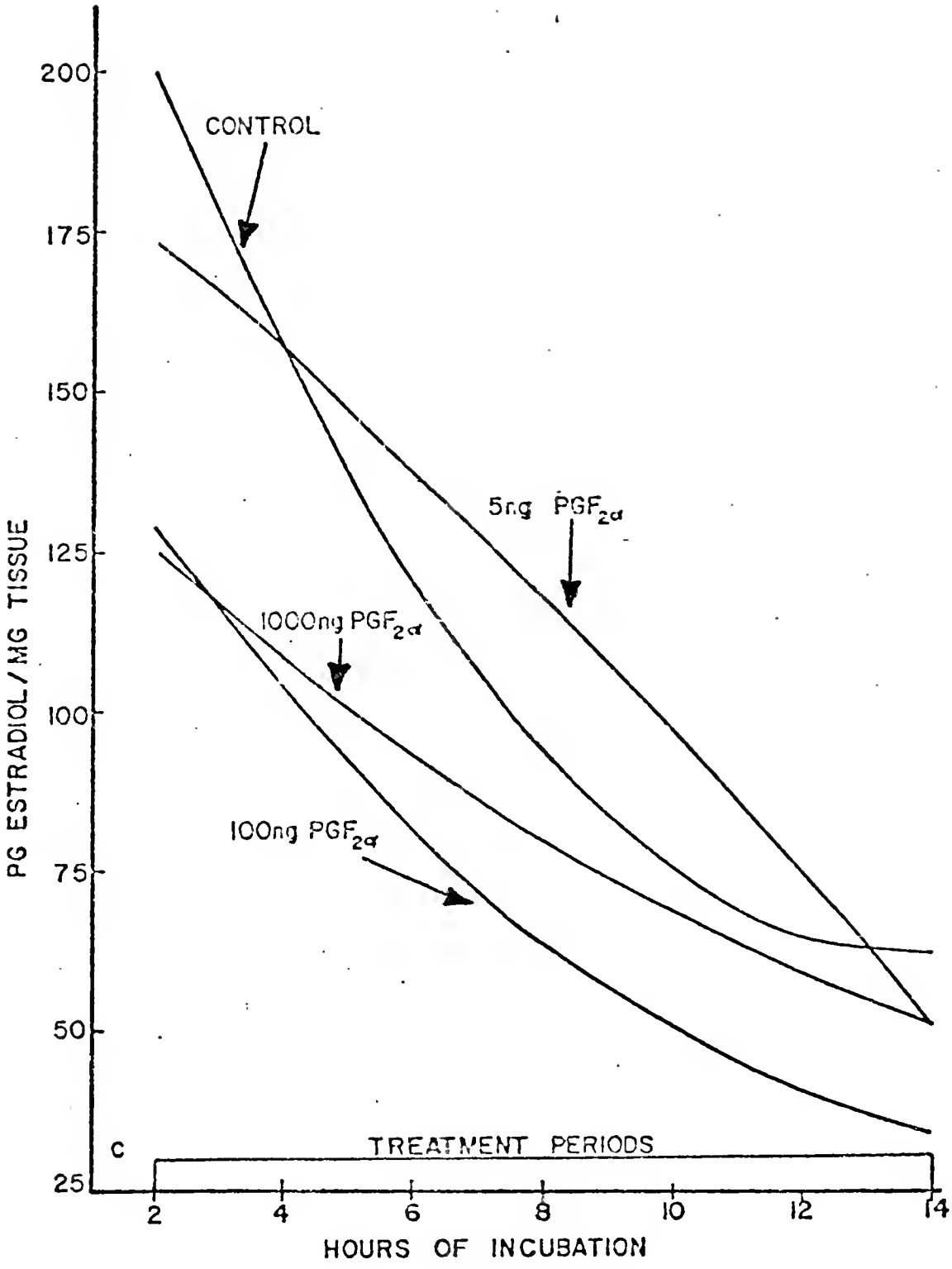


Figure 11. Effects of $PGF_{2\alpha}$ on in vitro estradiol secretion by bovine follicles (C = control period)

Hansel (1975a) who reported that 5 µg $PGF_{2\alpha}$ per ml medium failed to stimulate bovine follicular estradiol secretion in vitro. However, these workers observed that $PGF_{2\alpha}$ significantly stimulated testosterone secretion. In the previous study (Experiment 2, Section III) we failed to detect such an effect at a dose of 5 ng $PGF_{2\alpha}$ per ml medium, but testosterone was not measured in this experiment to evaluate the $PGF_{2\alpha}$ dose range. It is possible that $PGF_{2\alpha}$ stimulation of estradiol secretion observed in vivo (Chenault et al., 1976; Hixon et al., 1973) is a direct effect of $PGF_{2\alpha}$ on follicular steroidogenesis. If $PGF_{2\alpha}$ stimulates testosterone synthesis then higher concentrations of testosterone would be available to the granulosa cells for conversion to estrogens. It also is possible that the in vivo effect is an indirect action of $PGF_{2\alpha}$. Thorburn and Hales (1972) and Novy and Cook (1973) demonstrated that $PGF_{2\alpha}$ induced a shunting of blood from the corpus luteum to the stromal and follicular compartments of the ovary. This would increase the availability of nutrients and steroid precursors to follicles which may stimulate estrogen synthesis. Such a vascular response could not be detected with an in vitro incubation system involving just follicular components.

Experiment 4. Effect of FSH, Testosterone and FSH Plus Testosterone on In Vitro Estradiol Secretion by Bovine Follicles

In Experiment 2 (Section III) LH stimulated testosterone secretion, but estradiol secretion declined throughout the incubation. These results suggest that aromatase activity was inhibited or lost during incubation. In this experiment testosterone (5×10^{-7} molar), FSH (100 ng per ml medium) or testosterone and FSH together were added to the incubation medium to determine if aromatase enzymes were functional during incubation. Testosterone is a direct substrate for aromatization, and Dorrington et al. (1975) hypothesized that FSH has a direct stimulatory action on aromatase enzymes in the granulosa cells. Therefore, hormonal requirements for aromatization were supplied to the follicles in this experiment.

There was significant heterogeneity of regression between estradiol secretion curves (Figure 12). However, this is not necessarily a treatment effect. Estradiol secretion was appreciably higher during the control incubation before treatments in the FSH plus testosterone treatment group and fell at a faster rate than other groups during treatment periods. This resulted in a steeper decline of estradiol secretion when compared to the decline of estradiol secretion

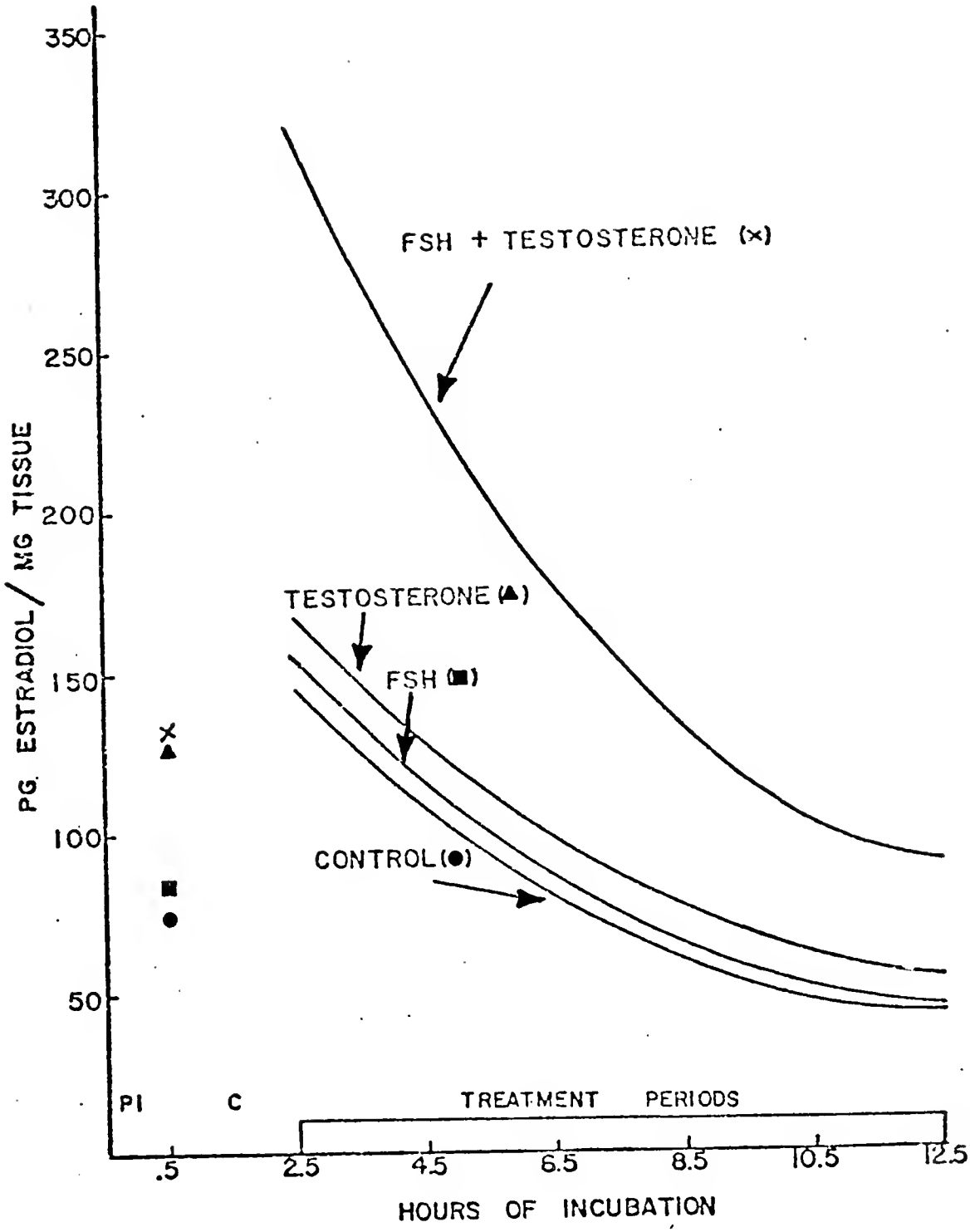


Figure 12. Effects of FSH, testosterone and FSH plus testosterone on in vitro estradiol secretion by bovine follicles (PI = preincubation period; C = control)

in the other three treatment groups. Consequently, the treatment secretion curves were not parallel. It was concluded that testosterone, FSH, or testosterone plus FSH had no effect on estradiol secretion in this incubation system when compared to controls.

These results do not supply evidence to support the hypothesis of Dorrington et al. (1975) that FSH stimulates estradiol secretion using the present incubation approach with FSH-p stimulated follicles. Dorrington et al. utilized immature hypophysectomized rats to show that granulosa cells required exposure to FSH for initiation of aromatase activity. It is not known if a continuous supply of FSH is needed to maintain estradiol secretion once it has been initiated. In contrast, large follicles from a mature, FSH-p treated cow were utilized in the present study. These follicles had been exposed to FSH in vivo before harvesting for incubation. Addition of LH, $PGF_{2\alpha}$, FSH, testosterone and FSH plus testosterone to incubation medium failed to stimulate estradiol secretion in these follicles (Experiments 2 to 4, Section III). Mills and Savard (1973) reported that in vitro incorporation of ^{14}C acetate into steroids could not be stimulated by LH if follicles were removed from rabbits 2 hr postcoitus, whereas incorporation was LH stimulated in follicles removed from estrus rabbits. These observations demonstrate that in vitro steroidogenesis was

maximally stimulated by endogenous gonadotropins released in response to mating. Such stimulation may reflect in vivo saturation of gonadotropin receptor sites. It is possible that the FSH-p induced follicles used in these studies have been stimulated by exogenous and endogenous gonadotropins prior to being harvested and therefore incapable of further response to any treatment. If the lack of response of follicles to treatments is due to maximal in vivo stimulation or receptor saturation then the addition of LH, FSH, and testosterone to the medium should have maintained a maximal stimulatory environment and estradiol secretion would have remained constant during incubation. This was not the case; estradiol secretion declined during incubation. This decline may be due to active inhibition or loss of aromatase activity by some biochemical action, disruption of the hypothesized bicellular estrogenic pathway, or cellular death. An alternate explanation could be that the follicles were atretic or luteinized before harvest and the decline in estradiol secretion observed in vitro mimicked what would have occurred in these follicles in vivo. Histological examination of follicles could support or eliminate some of these suggested explanations for the decline in estradiol secretion over time.

Experiment 5. Histology of Bovine Follicles Induced with FSH-p: Before or After In Vitro Incubation

In all preceding incubation experiments estradiol secretion declined during incubation and failed to respond to treatments applied in the incubation medium. Purpose of this experiment was to examine follicles for histological evidence to account for these results. Follicles from one FSH-p treated cow (10 to 15 mm) were placed in fixative after either isolation from the ovary (n=10) or a 14 hr incubation (n=5). To mimic structural relationships in incubated follicles some of the nonincubated follicles were punctured before being placed in fixative.

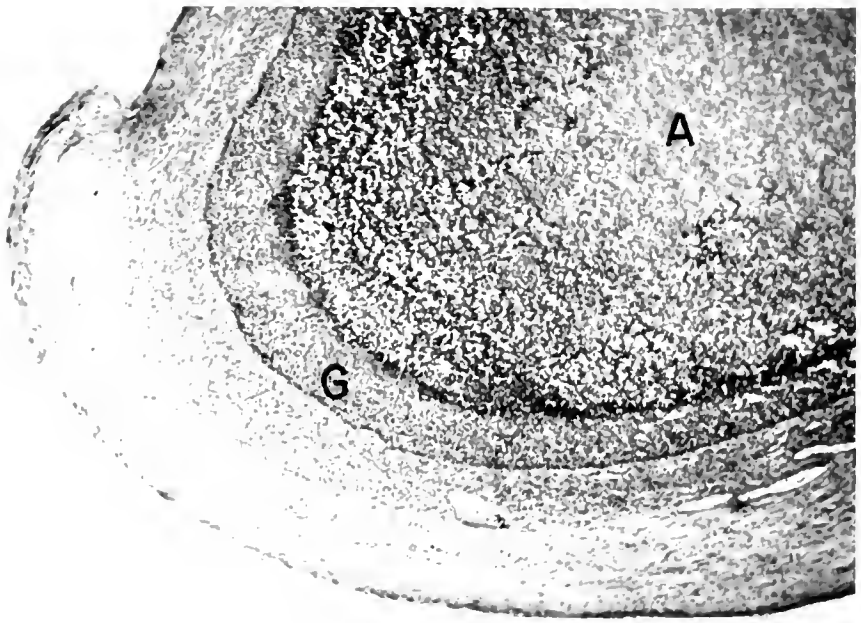
In all but one nonincubated follicle, the lamina propria was easily identified and the basal granulosa cell layer was distinct from the surrounding thecal layers (Figures 13 and 14). In one unincubated follicle the lamina propria was indistinct and demarcation between granulosa cell layers and theca layers was difficult to make. This may be a sign of early atresia in this follicle (Marion et al., 1968). However, a specific stain for lamina propria was not utilized in this study. Therefore the lamina propria may have been present in this follicle but not as distinct as in all other follicles. In all unincubated follicles the granulosa cell nuclei were plump (5.4±.77μm) and spherical

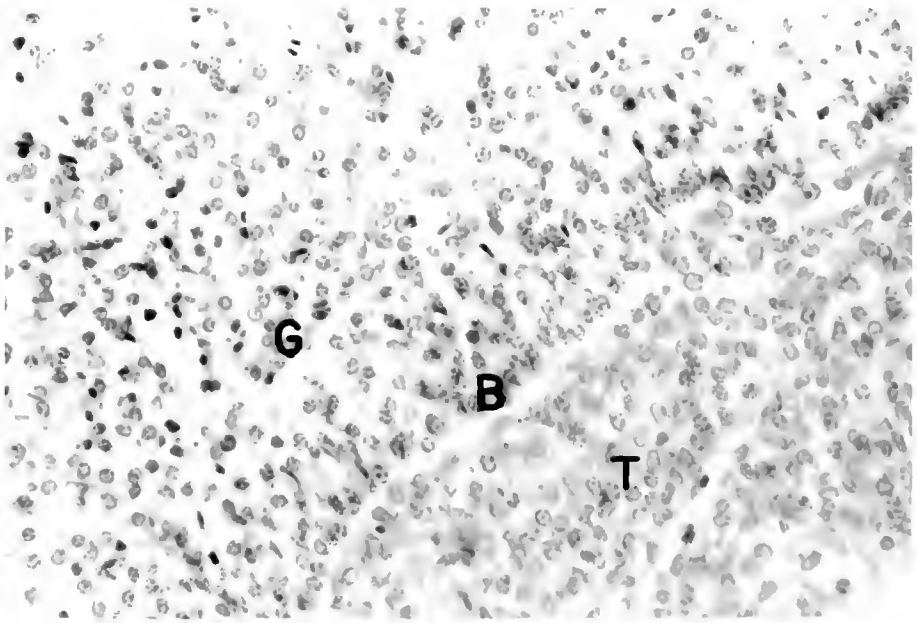
Figure 13. Portion of a nonincubated, FSH-p induced follicle (32X)

The follicular fluid in the antrum (A) is stained and surrounded by an intact layer of granulosa cells (G). The basal layer of granulosa cells is very distinct.

Figure 14. High power of a punctured, unincubated follicle (250X)

The basal layer of granulosa cells (B) is intact and demarks granulosa (G) and theca (T) cell layers.


A
G


G
B
T

and contained irregularly shaped areas of dark staining chromatin.

Within some follicles individual or clusters of granulosa cells were found free in the antrum and the granulosa cell layer was disrupted. This disruption in many cases appeared to be a result of mechanical dislocation during the fixation, embedding or sectioning procedures. Marion et al. (1968) identified the most frequently observed signs of early atresia in bovine follicles to be loosening and sloughing of the inner most (antral) layer of granulosa cells into the antrum and the presence of atretic bodies in the antrum. Atretic bodies are formed by the degradation of sloughed granulosa cells. No atretic bodies were observed in the present study and granulosa cells fround free in the antrum appeared normal. Large nonstaining droplets or vacuoles associated with the antral layer of granulosa cells also were observed (Figure 15). These droplets were distinguished easily from follicular fluid which was eosin stained. The nature or content of these droplets are unknown and have not been described previously in bovine follicles. Therefore they may be an artifact of the preservation procedure. However, these droplets may have been remnants of cells which had extruded their cytoplasm and nucleus. Droplets were associated with granules in the antrum which may have been remnants of such extruded material. These observations

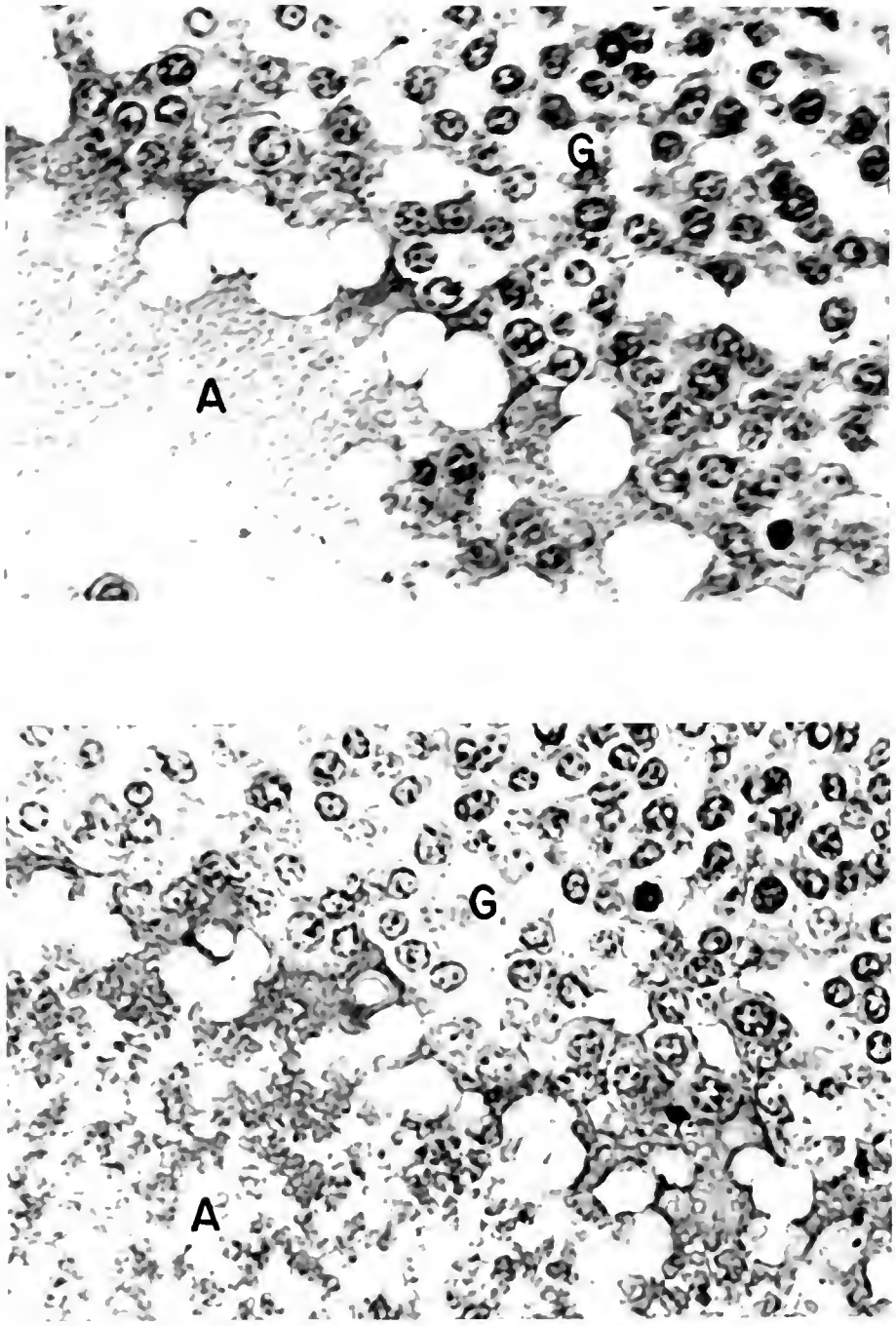


Figure 15. High power of a nonincubated follicle demonstrating vacuoles (400X)

Nonstaining vacuoles are associated with the antral layer of granulosa cells (G). In the lower picture granules are present in the antrum (A).

may be either signs of early atresia in some follicles, artifact of the fixation procedure or a result of mechanical disruption of the cells during follicle handling.

A theca layer was present; however, division into interna and externa was difficult to ascertain in intact follicles. Thecal layers became very distinguishable in punctured unincubated follicles. Once the pressure created by follicular fluid had been released in the punctured follicles the follicular walls collapsed and formed folds (Figures 14 and 16). Theca interna cells then assumed a rounded appearance whereas theca externa cells retained their fibroblastic or spindle shape. The theca interna cells and nuclei were very similar in appearance and staining characteristics to that described for granulosa cells. However, nuclei were not as concentrated in the theca interna layer suggesting that these cells were larger than granulosa cells.

In every follicle the vascular supply extended to, but never through, the lamina propria. This indicated that the follicles had not luteinized in vivo prior to harvest.

This description of FSH-p induced follicles is very similar to descriptions of nonatretic, bovine follicles published by Marion et al. (1968). In only one follicle was there clear evidence that atresia was occurring; however, early atresia may have been observed in several follicles. The mechanism by which FSH induces a greater than normal

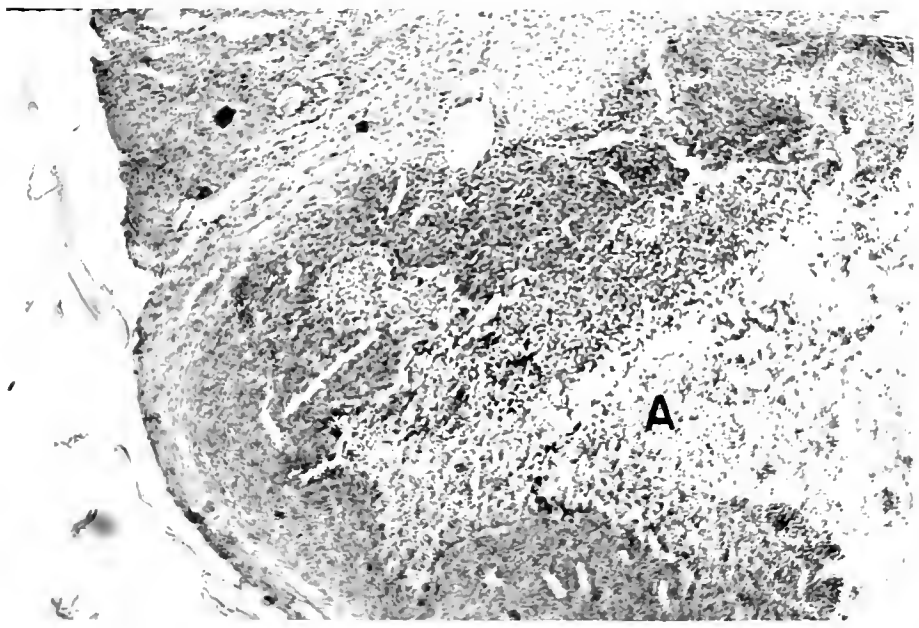


Figure 16. Portion of a punctured, unincubated follicle (32X)

Some granulosa cells have been disrupted and are free floating in the antrum (A). Follicular walls have collapsed and formed folds.

number of large follicles is unknown. Mechanisms may include inhibition of atresia, regeneration of atretic follicles and/or stimulation of growth in additional follicles. The histological evidence in this study sheds no light on the possible mechanism by which FSH induces follicular growth but does suggest that the FSH-p treatment used was a practical way to harvest large numbers of apparently normal Graafian follicles. This study also gives no insight into the intracellular biochemical nature of these unincubated follicles.

Despite trimming prior to incubation, follicles were surrounded with up to 10 layers of ovarian stromal connective tissue. In contrast to nonincubated follicles, incubated follicles showed considerable evidence of cellular degeneration. Nearly all granulosa cells were found in sheets, as individual cells or disrupted cells free floating within the antrum (Figures 17 and 18). Nuclei in all granulosa cells were shrunken (3.6±.41 m) and darker staining (Figure 19) than nuclei in nonincubated follicles. These are signs of pyknosis, an indication of cellular death. Similar observations have been made in swine follicles after being cultured free floating in medium for 8 days (Baker, Hunter and Neal, 1975).

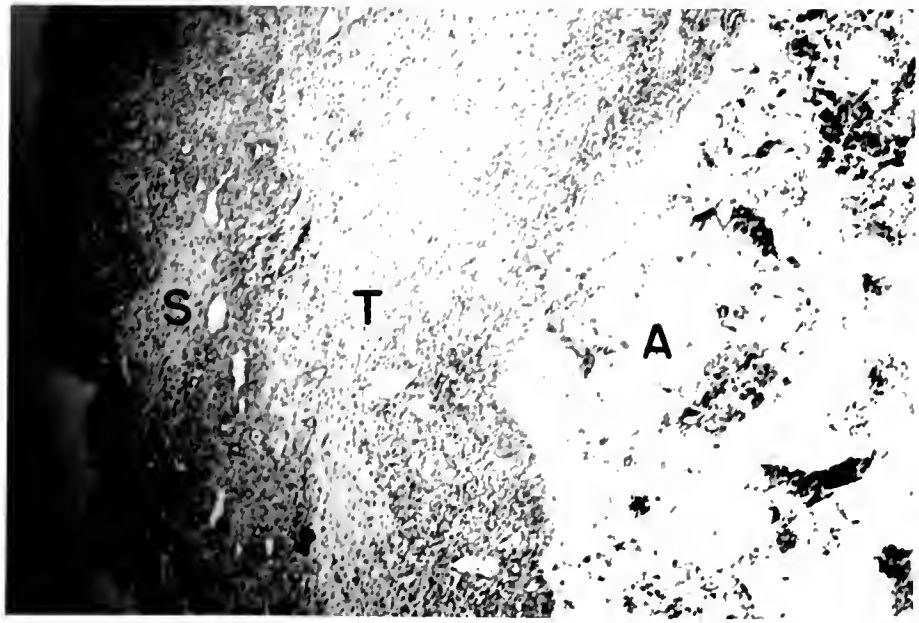
The theca interna and externa layers were intact and in apposition with each other (Figure 17). The outer thecal

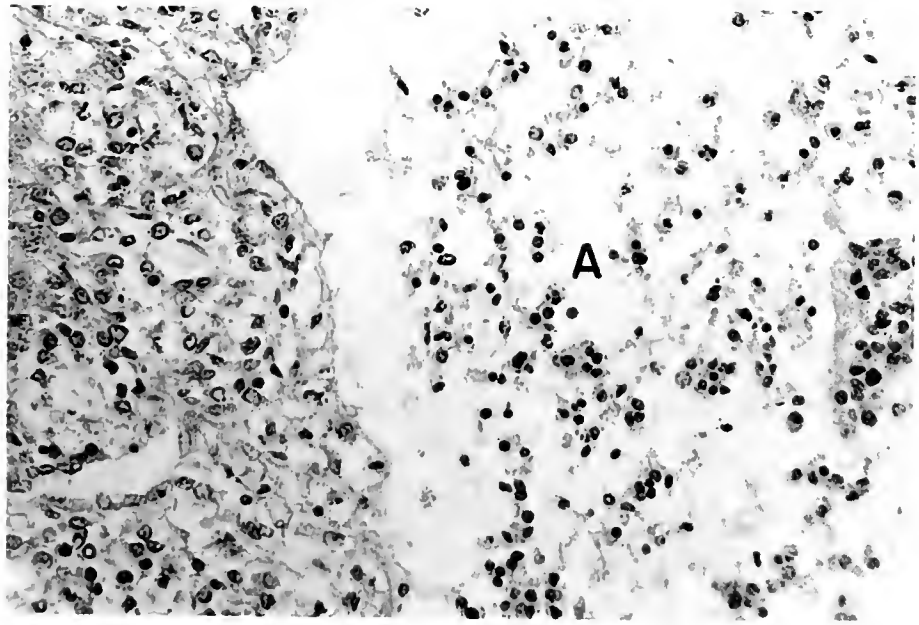
Figure 17. Portion of an incubated follicle (32X)

All granulosa cells are free floating in the antrum (A). The theca layer (T) and stroma (S) are in apposition with each other.

Figure 18. High power of an incubated follicle (250X)

All granulosa cells have been sloughed into the antrum (A) and are pyknotic.


S
T
A


A

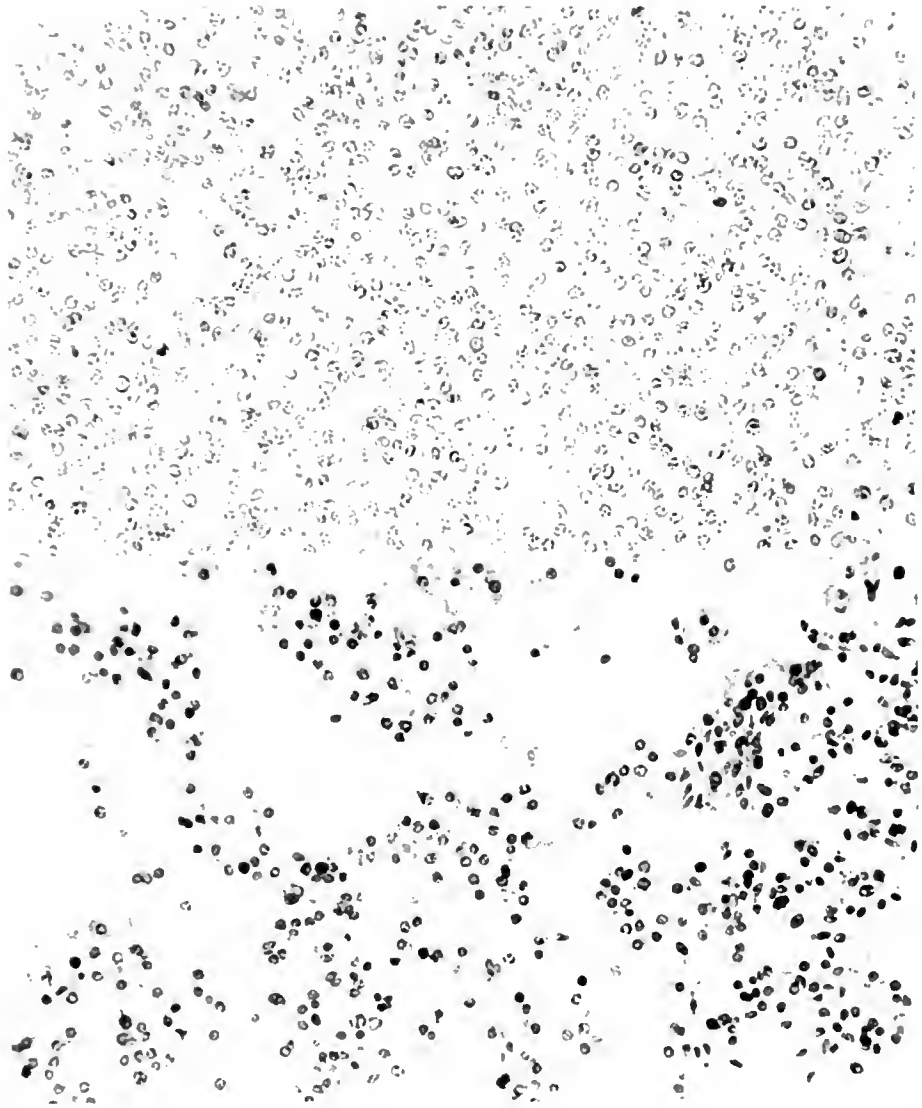


Figure 19. Comparison of granulosa cells in a punctured, nonincubated follicle (top) with pyknotic granulosa cells free floating in the antrum of an incubated follicle (bottom; 250X)

cell layers and stromal tissue appeared viable; however, pyknosis was evident in thecal layers located close to the antrum (Figure 20).

These results demonstrated that the incubation technique utilized for these studies was not able to maintain all cells over a 14 hr incubation. However, it was demonstrated (Experiment 2, Section III) that sufficient cells were viable early in incubation to produce a significant LH stimulation of testosterone and progesterone secretion. These observations provide several alternatives to explain the decline in estradiol secretion over time in the incubations. Undoubtedly some of this estradiol decline is a result of cell death during incubation as pyknosis was observed in all granulosa cells and the inner most theca cells after 14 hr of incubation. However, this would not account for the testosterone and estradiol secretion profiles observed in Experiment 2 (Section III) and it is difficult to determine when actual death occurred during incubation. Sloughing of granulosa cells into the antrum also may account for the decline in estradiol secretion. Sloughing would physically disrupt the hypothesized bicellular estrogen synthetic pathway. This may explain why LH stimulated testosterone secretion without any effect on estradiol secretion (Experiment 2, Section III). Testosterone may have been synthesized and released by theca cells in response

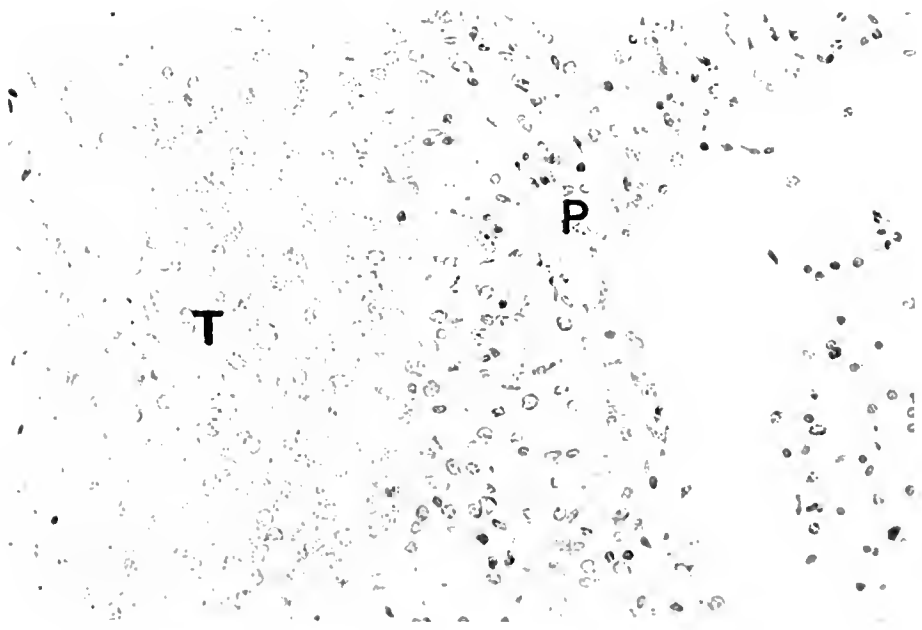


Figure 20. High power of an incubated follicle

Comparison of inner pyknotic theca cells (P) with viable outer theca cells (T).

to LH stimulation. However, testosterone could not be transported to granulosa cells for conversion to estradiol, therefore, testosterone accumulated in the medium.

This experiment also suggests that either bovine ovarian stromal tissue or theca cells synthesize progestin as progestin secretion was highest during the last periods of incubation (Experiment 2, Section III) when granulosa cells were pyknotic.

The cause of granulosa cell death in this incubation system is not known. Several factors may have contributed. Death may have resulted from a deficiency of nutrients or oxygen. Pyknosis was more evident in cells located farther from the medium (i.e., granulosa versus theca cells). Nutrients and oxygen would have a diffuse through several more cell layers, including up to 10 layers of stromal cells, than in vivo because an intact blood supply was not present. Cellular death may have been inhibited or slowed if additional stromal tissue had been removed before incubation.

It is not known if sloughing of the granulosa cells into the antrum is a cause or result of cell death. The granulosa layer was disrupted in unincubated follicles which had been punctured prior to fixation and the physical agitation during incubation may have caused further disruption. If the bicellular hypothesis of estradiol secretion is correct death and/or sloughing of granulosa cells could

account for the decline in estradiol secretion over time. If this hypothesis is not correct cell death in the theca layers could account for this decline.

This incubation procedure failed to maintain cells for the 14 hr of incubation. This observation does not affect interpretation of the incubation experiments. Cellular survival most likely could be improved if follicles were incubated as open sheets or tissue slices; however, these approaches would destroy the physiological integrity of follicular cell layers. Furthermore, Moor (1973) reported that sheep follicles cultured as opened flat sheets produced very low amounts of estrogens when compared to estrogen production by cultured whole follicles. Sheep (Moor, 1973; Moor et al., 1973) and swine (Baker et al., 1975) follicles have been cultured successfully for up to 8 days. In those organ culture systems the atmosphere was elevated above atmospheric pressure and follicles were supported on stainless steel grids to expose at least half of each follicle to the controlled atmosphere. The author recommends that future in vitro work with bovine follicles be conducted utilizing intact follicles in an organ culture system.

SECTION IV

SUMMARY AND CONCLUSIONS

Thirty-seven cycling dairy heifers were treated twice, 12 days apart, with 33.5 mg $PGF_{2\alpha}$ tham salt (IM) to test the feasibility of this management scheme to increase the percentage of animals in a potentially responsive phase of the estrous cycle at the second injection. Blood samples were collected from all animals prior to both $PGF_{2\alpha}$ injections and plasma was analyzed for progestin concentration.

On the first day of $PGF_{2\alpha}$ injection 68% of animals (25 of 37) were in a potentially responsive phase of the estrous cycle (not days 0 to 5). On any one day chosen at random 76% of cycling animals would be expected to be in a responsive phase of the cycle. In contrast, on the second injection day (12 days after the first injection) 36 of 37 animals (97%) were in a responsive phase of the cycle. This difference between distribution of heifers among the stages of the estrous cycle on the 2 days of injection was statistically significant ($P<.01$). Plasma progestin concentration was higher ($P<.01$) on the second injection day (6.51 ng per ml plasma) than on the first (3.33 ng per ml plasma)

indicating that more animals had a functional corpus luteum on the day of second injection and therefore were in the potentially responsive luteal phase of the estrous cycle. Sixty percent of animals returned to estrus within 8 days following the first injection whereas 89% responded with estrus within 8 days after the second injection.

In conclusion these data clearly demonstrated that two injections of $PGF_{2\alpha}$ (33.5 mg; IM) is a practical management scheme to increase the percentage of animals responsive to the second injection. However, due to the large variation in time of onset of estrus following the second injection (8 days), these data indicate that a single predetermined insemination would not be practical.

Two experiments were conducted to determine the effect of $PGF_{2\alpha}$ on heart rate, arterial blood pressure, uterine temperature and arterial blood temperature in dairy cows. To monitor these biological systems a thermocouple was placed in the uterine serosa and an additional thermocouple and catheter placed into the external iliac artery of 2 cows.

In the first experiment animals (n=2) received an IM injection of saline (5 ml) followed several hours later by IM injection of 33.5 mg $PGF_{2\alpha}$ tham salt. IM injection of either saline (n=3) or $PGF_{2\alpha}$ (n=3) evoked a transient increase in heart rate and blood pressure. The increased heart rate lasted less than 2 min following both treatments.

Following saline injection blood pressure returned to pretreatment levels by 2 min posttreatment, whereas it remained slightly elevated above pretreatment values following $PGF_{2\alpha}$. No alteration in uterine or arterial blood temperatures were detected following either treatment. Since observed effects following saline or $PGF_{2\alpha}$ were similar these responses were attributed to route of administration and not to a drug effect. In contrast, immediate, dramatic physiological responses were observed following intravenous infusions (n=2; both in the same animal) of 33.5 mg $PGF_{2\alpha}$ via a jugular catheter in the second experiment. Heart rate dropped from 34 to 16 beats per 30 sec and arterial blood pressure rose from 140 to 230 mm of Hg within the first min after $PGF_{2\alpha}$ infusion. Uterine temperature increased abruptly and the difference between uterine and arterial blood temperature (ΔT_{u-A}) widened immediately following infusion. These were followed by a slow decline in arterial blood temperature (.4 C) and a corresponding decline in uterine temperature. Intravenous infusion of saline caused no detectable affect on any of the biological systems monitored. The elevated blood pressure following $PGF_{2\alpha}$ was due most likely to a $PGF_{2\alpha}$ induced vasoconstriction, whereas the decline in heart rate probably was a physiological response to elevated blood pressure and not a direct effect of $PGF_{2\alpha}$. Inhibition of heat loss resulting from a $PGF_{2\alpha}$ induced reduction in uterine

blood flow may account for the increased difference between uterine and arterial blood temperature following $PGF_{2\alpha}$ infusion. The subsequent decline in uterine and arterial blood temperatures reflects a loss of body temperature possibly by $PGF_{2\alpha}$ induced peripheral vesodialation.

To determine if the increased ΔT_{u-A} was due to decreased uterine blood flow induced by $PGF_{2\alpha}$, electromagnetic blood flow transducers were fitted around one miduterine artery in three ovariectomized sheep. To increase uterine blood flow ewes were administered 20 µg estradiol intravenously 2 hr prior to intravenous injection of 8 mg $PGF_{2\alpha}$. Uterine blood flow decreased from 72 to 43 ml per min within the first min after infusion and remained depressed for up to 60 min postinjection. This provides direct evidence that $PGF_{2\alpha}$ reduces blood flow to the uterus.

In conclusion these experiments demonstrated that IM injection is a safe, practical way to administer a luteolytic dose of $PGF_{2\alpha}$. In contrast intravenous infusion of the same dose of $PGF_{2\alpha}$ induced rapid biological responses which may prove detrimental to cattle.

For the final series of experiments an in vitro incubation system was established to study the direct effects of various hormonal treatments on bovine ovarian follicular steroidogenesis. To obtain sufficient numbers of follicles for incubation, cows were treated two times daily with FSH-p

from days 16 to 19 of the estrous cycle and ovariectomized on day 20. After separation from the ovary follicles were trimmed of connective tissue and follicular fluid aspirated. Follicles were then weighed and assigned randomly to treatments. Follicles were incubated individually in 5 ml medium, at 37 C, under a gaseous atmosphere of 50% N_2, 45% O_2 and 5% CO_2. Incubation media was changed every 2 hr to be able to determine hormonal secretory profiles over time. Various hormonal treatments were added directly to the media. Treatment effects and time trends of steroid secretion during incubation were analyzed by least squares analyses.

Following preparation for incubation follicles were either immediately frozen (n=5) or incubated for 14 hr (n=5) in the first experiment. Incubated follicles secreted significantly more ($P<.01$) estradiol into the medium (64.3 pg per mg) during the 14 hr incubation than could be extracted from incubated follicular tissue (16.1 pg per mg). This indicated that bovine follicles synthesized estradiol in vitro however estradiol secretion declined during incubation.

In the second experiment $PGF_{2\alpha}$ (5 ng per ml medium) and LH (50 ng per ml medium) had no effect on in vitro estradiol secretion and estradiol secretion declined during incubation. Progestin secretion increased over time in the control follicles and LH stimulated progestin secretion. Progestin secretion in the $PGF_{2\alpha}$ group had no significant time trend during

incubation. In all three groups testosterone secretion increased in the pretreatment periods and then declined in the control and $PGF_{2\alpha}$ groups during the treatment periods. This initial increase in testosterone secretion occurred when estradiol secretion was declining. This suggests that activity of the aromatase enzyme may be lost early in the incubation. In contrast to the decline in other treatment groups, LH stimulated testosterone secretion. The stimulatory action of LH on progestin and testosterone secretion identifies the bovine as another species in which LH stimulates steroidogenesis.

$PGF_{2\alpha}$ at concentrations of 5, 10 or 1000 ng were added per ml of media in the third experiment. These higher concentrations of $PGF_{2\alpha}$ had no effect on estradiol secretion in vitro.

In the final incubation experiment FSH (100 ng per ml) testosterone (5×10^{-7}M) or FSH plus testosterone were added to the incubation medium. These treatments provided follicles with all the known hormonal requirements for aromatization but again estradiol secretion declined over time and treatments had no effect on estradiol secretion. These results suggested that the aromatase enzyme was inhibited or lost during incubation.

Incubated and nonincubated follicles were examined for histological evidence to account for the incubation results in the final experiment. FSH-p induced, nonincubated

follicles were observed to be normal, mature, Graafian follicles. Following 14 hr of incubation severe cellular disruption and death were observed. Granulosa cells had been sloughed into the antrum and nuclei of all granulosa cells and inner (antral) theca cells were pyknotic. These observations suggest that the decline in estradiol secretion observed in the incubation studies was due to disruption of the hypothesized bicellular estradiol synthetic pathway via disassociation of granulosa cells from theca cells and/or death of granulosa and theca cells.

In conclusion this incubation system failed to maintain follicular cells during 14 hr of incubation. Therefore this system is not recommended for in vitro studies. Organ culture systems have been used successfully by other workers to maintain follicles in vitro. Therefore organ cultures are suggested as an alternative for future work in this area.

APPENDICES

APPENDIX 1

MEDIUM 199

Table 6. Medium 199 ingredients per liter

L-Arginine	70 mg	Hypoxanthine	.3 mg
L-Histidine	20 mg	Thymine	.3 mg
L-Lysine	70 mg	Uracil	.3 mg
L-Tyrosine	40 mg	Thiamine	.01 mg
DL-Tryptophane	20 mg	Riboflavin	.01 mg
DL-Phenylalanine	50 mg	Pyridoxine	.025 mg
L-Cystine	20 mg	Pyridoxal	.025 mg
DL-Methionine	30 mg	Niacin	.025 mg
DL-Serine	50 mg	Niacinamide	.025 mg
DL-Threonine	60 mg	Pantothenate	.01 mg
DL-Leucine	120 mg	Biotin	.01 mg
DL-Isoleucine	40 mg	Folic acid	.01 mg
DL-Valine	50 mg	Choline	.5 mg
DL-Glutamic acid	150 mg	Inositol	.05 mg
DL-Aspartic acid	60 mg	p-Aminobenzoic acid	.05 mg
DL-Alanine	50 mg	Vitamin A	.1 mg
L-Proline	40 mg	Calciferol	.1 mg
L-Hydroxyproline	10 mg	Menadione	.01 mg
Glycine	50 mg	a-Tocopherol phosphate	.01 mg
L-Cysteine	.1 mg	Ascorbic acid	.05 mg
Adenine	10 mg	Glutathione	.05 mg
Guanine	.3 mg	Cholesterol	.2 mg
Xanthine	.3 mg		

L-Glutamine	100 mg
Adenosine triphosphate	1 mg
Adenylic acid	.2 mg
Ribose	.5 mg
Desoxyribose	.5 mg
Bacto-dextrose	1 g
Tween 80	5 mg
Sodium acetate	50 mg
Iron (ferric nitrate)	.1 mg
Sodium chloride	8 g
Potassium chloride	.4 g
Calcium chloride	.14 g
Magnesium sulfate	.2 g
Disodium phosphate	60 mg
Monopotassium phosphate	60 mg
Sodium bicarbonate	.35 g
Bacto-phenol red	20 mg
Triple distilled water	1000 ml

APPENDIX 2

HISTOLOGICAL SOLUTIONS AND PROCEDURES

Table 7. Bouins fixation solution

Picric acid (saturated aqueous solution)	75 ml
Formalin	25 ml
Glacial acetic acid	5 ml

Table 8. Dehydration, dealcoholization and infiltration procedures

Dehydration	
Percent ethanol	Time (hr)
30	1
50	1
70	2
80	12 (overnight)
100	2
100	2

Dealcoholization	
Ethanol:xylene	Time (hr)
2:1	1
1:1	1
1:2	1
0:1	12 (overnight)
0:1	1

Infiltration	
Solution	Time (hr)[a]
Xylene saturated with paraffin	2
Paraffin	2
Paraffin	2[b]

[a]Time in oven at 60 C.

[b]Cool overnight at room temperature before sectioning.

Table 9. Hematoxylin and eosin staining procedure

Solution	Time (min)
Xylene	2
Xylene	2
Ethanol	2
Ethanol	2
Water (distilled)	2
Hematoxylin	4
Water (distilled)	2
Water (distilled) plus five drops concentrated NH_3	2
Water (tap)	2
Eosin-phloxine	2
Ethanol	2
Ethanol	2
Xylene	2
Xylene	4

APPENDIX 3

HORMONAL DATA AND STATISTICAL ANALYSES

Table 10. Estradiol secretion (pg per mg tissue) by incubated bovine follicles in Experiment 1, Section III

Follicle	Weight (mg)	Hours of incubation						
		2	4	6	8	10	12	14
1	212.1	9.51	14.83	11.56	11.17	6.78	6.31	3.60
2	152.9	9.81	16.24	9.01	9.49	6.66	5.53	4.97
3	117.7	8.92	10.48	10.93	8.25	5.70	4.30	5.05
4	188.6	11.05	11.96	11.98	10.15	6.66	12.55	3.63
5	134.4	11.95	15.64	13.69	11.89	6.99	9.50	4.80
Mean	161.2	10.26	13.83	11.43	10.19	6.56	7.64	4.41

Table 11. Estradiol secretion (pg per mg tissue) by bovine follicles in Experiment 2, Section III.

Control follicles	Weight (mg)	Hours of incubation						
		2[a]	4[b]	6[c]	8[c]	10[c]	12[c]	14[c]
1	257.0	94.33	92.59	47.70	45.97	40.00	26.00	24.20
2	165.0	63.15	68.25	59.34	52.27	45.58	40.07	21.45
3	232.8	256.90	111.80	125.30	88.40	115.10	48.00	43.60
4	121.3	240.77	151.70	87.34	63.93	89.83	106.07	76.52
5	213.0	60.86	61.54	50.81	43.57	31.80	30.71	32.39
Mean	197.8	143.20	97.18	74.10	58.83	58.93	37.99	33.22
$PGF_{2\alpha}$ follicles								
6	259.6	106.52	138.76	112.76	67.95	67.78	55.70	59.40
7	184.5	155.12	135.51	111.38	68.11	57.22	52.39	41.62
8	202.3	101.57	78.71	76.80	65.97	59.67	36.81	34.32
9	102.0	367.63	266.30	213.50	161.51	104.71	84.90	90.54
10	101.0	136.40	130.10	129.44	99.51	83.30	68.43	52.99
Mean	169.8	173.45	136.34	129.56	94.01	74.54	59.65	55.77
LH follicles								
11	126.3	185.67	153.30	121.14	128.39	69.23	---	41.96
12	228.3	57.49	52.27	37.43	49.58	39.85	46.16	38.55
13	197.1	157.52	183.48	129.04	140.38	85.56	71.80	40.89
14	205.9	120.56	71.42	84.31	66.97	49.36	45.32	39.93
15	90.8	197.14	163.30	112.31	87.52	57.95	47.36	36.52
Mean	169.6	143.68	124.75	96.85	94.57	60.39	52.66	38.77

[a]Preincubation

[b]Control incubation

[c]Treatment periods

Table 12. Progestin secretion (pg per mg tissue) by bovine follicles in Experiment 2, Section III

Control follicles	Hours of incubation						
	2[a]	4[b]	6[c]	8[c]	10[c]	12[c]	14[c]
1	--	108.25	107.66	116.30	93.46	96.34	140.38
2	46.91	33.50	49.38	41.89	80.65	105.10	115.14
3	19.17	31.30	36.38	47.06	51.91	48.10	55.15
4	57.92	66.13	64.62	96.53	89.04	124.28	105.62
5	54.87	37.32	54.97	73.02	89.25	104.26	133.98
Mean	44.72	55.72	62.60	74.96	80.86	95.62	102.85
$PGF_{2\alpha}$ follicles							
6	278.51	148.93	147.65	92.07	72.29	108.52	100.68
7	150.47	94.61	139.03	129.80	202.97	186.34	156.72
8	47.30	18.51	38.72	45.86	74.84	71.75	94.48
9	20.67	29.66	39.33	68.06	64.54	45.96	63.19
10	88.37	108.48	123.09	68.37	78.68	95.69	258.75
Mean	117.06	80.04	97.56	80.83	88.58	100.65	134.76
LH follicles							
11	258.75	99.10	105.18	182.10	107.51	144.62	167.88
12	39.14	58.59	66.42	161.59	155.08	127.50	121.87
13	128.09	63.87	106.18	163.94	139.87	--	124.00
14	125.91	51.76	129.85	173.24	184.87	116.63	138.78
15	24.23	41.16	49.37	106.44	98.22	90.31	75.44
Mean	115.24	62.90	91.40	157.46	133.11	121.80	125.59

[a]Preincubation

[b]Control incubation

[c]Treatment periods

Table 13. Testosterone secretion (pg per mg tissue) by bovine follicles in Experiment 2, Section III

Control follicles	Hours of incubation						
	2[a]	4[b]	6[c]	8[c]	10[c]	12[c]	14[c]
1	--	127.43	105.29	100.34	85.90	65.21	54.94
2	16.92	27.22	29.12	42.80	38.84	31.25	26.13
3	26.19	76.07	107.19	118.92	89.83	106.06	76.52
4	86.86	129.82	103.61	93.87	98.47	65.24	46.70
5	44.83	63.15	60.0]	53.67	47.05	57.76	35.77
Mean	44.45	84.74	81.05	81.92	72.02	65.10	48.01
$PGF_{2\alpha}$ follicles							
6	182.40	309.40	222.07	198.50	170.11	127.54	110.21
7	200.59	351.97	353.38	380.65	344.06	286.28	249.59
8	203.81	240.88	183.34	155.56	125.56	107.96	104.94
9	94.31	150.78	140.59	131.18	95.49	73.53	301.57
10	316.82	396.29	288.28	301.55	281.94	169.66	115.65
Mean	199.59	289.86	237.53	233.49	203.43	152.99	176.39
LH follicles							
11	75.61	191.04	228.65	437.02	430.69	392.29	298.71
12	18.83	23.57	47.74	59.26	53.71	56.59	58.48
13	116.74	277.53	386.05	428.47	601.37	421.31	451.70
14	52.40	100.39	154.30	277.22	323.60	253.71	222.78
15	96.26	290.53	237.44	310.46	320.26	341.41	211.89
Mean	71.97	176.61	210.84	302.49	345.91	293.06	248.71

[a]Preincubation

[b]Control incubation

[c]Treatment period

Table 14. Estradiol secretion (pg per mg tissue) by bovine follicles in Experiment 3, Section III

Control follicles	Weight (mg)	Hours of incubation						
		2[a]	4[b]	6[b]	8[b]	10[b]	12[b]	14[b]
1	152.8	421.63	178.78	255.21	137.42	99.14	92.46	96.26
2	111.8	295.70	200.50	170.70	143.80	98.10	97.00	94.00
3	249.9	80.60	76.70	64.00	58.00	57.70	29.09	29.85
4	127.4	188.69	146.93	153.92	89.71	83.51	80.53	62.79
5	121.5	64.61	60.33	49.38	41.73	41.48	30.20	30.04
Mean	152.7	210.25	133.25	138.64	94.13	75.99	65.86	62.59
5 ng $PGF_{2\alpha}$ follicles								
6	131.6	413.77	339.30	378.58	204.57	211.10	139.67	86.86
7	117.3	158.99	142.62	151.57	100.08	95.05	72.12	81.07
8	119.0	106.30	104.03	99.74	66.89	59.91	38.65	38.99
9	152.2	96.70	102.23	77.72	110.31	75.82	55.85	40.27
10	113.5	83.62	70.75	54.01	65.73	51.28	48.72	31.98
Mean	126.7	171.89	151.79	152.32	109.52	99.23	71.00	55.83

[a]Preincubation

[b]Treatment periods

Table 14 (continued)

100 ng $PGF_{2\alpha}$ follicles	Weight (mg)	Hours of incubation						
		2[a]	4[b]	6[c]	8[c]	10[c]	12[c]	14[c]
11	177.3	205.92	155.44	89.56	72.98	78.06	48.05	38.80
12	226.3	124.00	127.89	86.21	58.24	54.13	26.12	19.58
13	112.2	149.29	155.98	134.05	104.46	84.67	74.16	58.02
14	136.7	38.11	44.18	42.86	57.86	33.06	28.38	59.25
15	123.2	114.53	73.94	43.18	26.22	16.32	6.33	9.09
Mean	155.1	126.37	111.49	79.17	63.95	53.25	36.61	36.95
1000 ng $PGF_{2\alpha}$ follicles								
16	139.2	187.57	142.39	140.45	108.77	93.61	68.75	65.09
17	164.8	128.76	119.96	105.76	133.31	60.74	75.36	63.59
18	174.2	112.01	74.81	88.30	81.29	69.98	57.58	62.75
19	104.0	112.78	100.77	58.07	74.71	39.90	45.86	40.38
20	99.8	94.93	80.02	63.08	55.68	45.84	43.61	32.25
Mean	136.4	127.21	103.59	91.13	90.75	62.01	58.23	52.81

Table 15. Estradiol secretion (pg per mg tissue) by bovine follicles in Experiment 4, Section III

Control follicles	Weight (mg)	Hours of incubation						
		.5[a]	2.5[b]	4.5[c]	6.5[c]	8.5[c]	10.5[c]	12.5[c]
1	114.6	70.53	147.00	123.17	88.34	72.65	45.86	42.42
2	120.3	48.03	145.75	85.05	66.07	38.34	35.27	31.01
3	94.4	92.21	220.95	174.66	140.25	106.68	94.59	93.24
4	105.5	90.16	84.97	57.70	49.32	33.84	28.86	16.39
5	50.0	69.56	154.11	71.62	74.44	55.75	35.77	31.82
Mean	97.0	74.10	150.56	102.44	83.68	61.45	48.06	42.98
Testosterone follicles								
6	lost	--	--	--	--	--	--	--
7	131.6	250.23	161.50	104.83	82.36	54.18	64.24	40.93
8	123.4	108.20	240.61	190.76	141.37	145.65	122.54	117.58
9	89.5	113.66	139.32	82.30	53.34	32.38	19.39	15.11
10	69.9	37.89	130.37	140.21	111.80	75.16	44.99	38.31
Mean	82.9	127.5	167.95	129.53	97.22	76.84	62.79	52.98

[a]Preincubation

[b]Control incubation

[c]Treatment periods

Table 15 (continued)

FSH follicles	Weight (mg)	Hours of incubation						
		.5[a]	2.5[b]	4.5[c]	6.5[c]	8.5[c]	10.5[c]	12.5[c]
11	131.0	88.82	203.94	149.69	102.24	86.11	71.48	51.09
12	93.4	145.34	284.31	206.66	140.13	94.68	87.19	71.75
13	85.7	65.66	113.61	117.78	89.71	56.74	59.03	36.93
14	61.7	72.94	147.30	102.33	72.03	58.07	57.56	50.67
15	86.9	49.71	33.07	19.90	11.96	10.12	6.31	5.56
Mean	91.7	84.49	156.45	119.27	83.21	61.14	56.31	43.20
FSH & Testosterone follicles								
16	139.5	203.06	693.62	451.94	388.25	278.53	240.12	214.45
17	96.0	195.78	332.63	242.71	175.01	165.13	137.91	82.13
18	92.8	113.26	288.08	192.13	132.52	103.48	57.16	32.79
19	83.2	100.21	236.20	173.93	116.51	101.08	72.75	48.85
20	73.0	48.45	96.76	79.89	62.71	46.04	56.89	24.20
Mean	96.9	132.15	329.46	228.12	165.00	138.85	112.97	80.48

Table 16. Analysis of variance for estradiol secretion in Experiment 1, Section III

Source	df	SS for pg estradiol per mg tissue	SS for total estradiol (ng)
Total	34	400.369	15.246
Follicle	4	33.042*	4.776**
Time			
Linear	1	39.627	1.128
Quadratic	1	34.409	.992
Cubic	1	28.136	.827
Quartic	1	24.010**	.720**
Remainder	26	78.742	2.776

*($p < .05$)
**($p < .01$)

Table 17. Analysis of variance for estradiol secretion (pg for mg tissue) in Experiment 2, Section III

Source	Pooled df	Pooled SS	Control df	Control SS	$PGF_{2\alpha}$ df	$PGF_{2\alpha}$ SS	LH df	LH SS
Total	103	1161587.69	34	269889.19	35	542249.21	34	349449.29
Mean	1	801427.37	1	169822.28	1	386978.29	1	268547.01
Treatment	2	18120.86						
Foll (control)	4	26816.12	4	26700.24**				
Foll (PGF)	4	60560.06			4	60560.07**		
Foll (LH)	4	24692.06					4	24787.60**
Time								
Linear	1	21373.58	1	10778.30	1	8235.86	1	3372.30
Quadratic	1	4789.47*	1	4290.16*	1	1527.58	1	223.31
Remainder	86	77350.06	27	26950.32	28	31504.16	27	16223.17

Test of heterogeneity of regression

Source	df	Remainder SS	MS
Pooled	86	77350.06	899.42
Sum of treatments	82	74677.65	910.70
Difference	4	2672.41	668.10

$$F = \frac{668.10}{910.70} = .734^{NS}$$

**P < .01
*P < .05
NS - nonsignificant

Table 18. Analysis of variance for progestin secretion (pg for mg tissue) in Experiment 2, Section III

Source	Pooled df	Pooled SS	Control df	Control SS	$PGF_{2\alpha}$ df	$PGF_{2\alpha}$ SS	LH df	LH SS
Total	103	1194673.60	34	231111.4	35	432694.5	34	530867.7
Mean	1	951938.95	1	199013.81	1	328876.7	1	448665.8
Treatment	2	25096.58						
Foll (control)	4	16733.08	4	15045.10**				
Foll ($PGF_{2\alpha}$)	4	59671.54			4	59671.5**		
Foll (LH)	4	26678.83					4	26600.8**
Time								
Linear	1	9223.16	1	12.23	1	1309.8	1	14916.0
Quadratic	1	8437.66	1	21.67	1	941.5	1	14288.9
Cubic	1	7239.06	1	16.90	1	718.6	1	12645.8
Quartic	1	6228.61*	1	16.51	1	583.2	1	10969.0
Remainder	84	95808.02	25	6174.70	26	41170.7	25	29865.0**

Test for heterogeneity of regression

Source	df	Remainder SS	MS
Pooled	84	95808.02	1140.57
Sum of treatments	76	77210.4	1015.90
Difference	8	18597.62	2324.7

$$F = \frac{2324.7}{1015.9} = 2.29^*$$

**P < .01
*P < .05

Table 19. Analysis of variance for testosterone secretion (pg per mg tissue) in Experiment 2, Section III

Source	Pooled df	Pooled SS	Control df	Control SS	$PGF_{2\alpha}$ df	$PGF_{2\alpha}$ SS	LH df	LH SS
Total	103	4675858.96	34	195581.06	35	1894763.96	34	2585513.94
Mean	1	3025878.05	1	162883.62	1	1592975.65	1	1832581.41
Treatment	2	553464.29*						
Foll (control)	4	18069.15	4	19475.68**				
Foll ($PGF_{2\alpha}$)	4	145540.94			4	145540.94		
Foll (LH)	4	430030.60					4	435277.31**
Time								
Linear	1	86374.86	1	3859.89	1	3157.31	1	146665.16
Quadratic	1	77593.66**	1	4818.87**	1	8969.58	1	97032.24**
Remainder	86	424316.97	27	9083.46	28	122329.18	27	110302.10

Test for heterogeneity of regression

Source	df	Remainder SS	MS
Pooled	86	424316.97	4085.26
Sum of treatments	82	241714.74	2947.74
Difference	4	182602.23	45650.55

$$F = \frac{45650.55}{2947.74} = 15.486^{**}$$

**P < .01
*P < .05

Table 20. Analysis of variance for estradiol secretion (pg per mg tissue) in Experiment 3, Section III

Source	Pooled df	Pooled SS	Control df	Control SS	5 ng $PGF_{2\alpha}$ df	5 ng $PGF_{2\alpha}$ SS	100 ng $PGF_{2\alpha}$ df	100 ng $PGF_{2\alpha}$ SS	1000 ng $PGF_{2\alpha}$ df	1000 ng $PGF_{2\alpha}$ SS
Corrected total	139	696851.48	34	238805.71	34	289663.95	34	79253.05	34	42400.88
Treatment	3	46727.90								
Foll (treatment)	16	320883.45**	4	102144.52**	4	177511.08**	4	26583.80**	4	14644.05**
Time										
Linear	1	176703.84	1	72953.31	1	1431.75	1	6191.96	1	2419.87
Quadratic	1	4217.72*	1	7033.90	1	164.45	1	1591.63[a]	1	359.03
Remainder	118	148318.58	28	56663.98	28	55321.24	28	15883.32	28	6385.62

Test of heterogeneity of regression

Source	df	Remainder SS	MS
Pooled	118	148318.58	
Sum of treatments	112	134254.16	1198.70
Difference	6	14064.42	2344.07

$$F = \frac{2344.07}{1198.70} = 1.955^{a}$$

**$p < .01$
*$p < .05$
[a]$p > .05$

Table 21. Analysis of variance for estradiol secretion (pg per mg tissue) Experiment 4, Section III.

Source	Pooled df	Pooled SS	Control df	Control SS	Testosterone df	Testosterone SS	FSH df	FSH SS	FSH + Testosterone df	FSH + Testosterone SS
Corrected total	113	1071805.58	29	72705.40	23	75938.38	29	120810.04	29	621207.27
Treatment	3	181144.47								
Foll (treatment)	15	472226.56**	4	29684.75**	4	34414.69**	4	57438.97**	4	350688.15**
Time Linear	1	47618.14**	1	7292.43	1	5930.33	1	8524.15	1	31962.37
Quadratic	1	18916.78**	1	3083.87**	1	2223.93**	1	3579.10*	1	12226.47*
Remainder	93	134584.15	23	3495.03	18	3146.10	23	16649.15	23	69332.86

Test of heterogeneity of regression

Source	df	Remainder SS	MS
Pooled	93	134584.15	1447.14
Sum of treatments	87	92623.14	1064.63
Difference	6	41961.01	6993.50

$$F = \frac{6993.50}{1064.63} = 6.569^{**}$$

** P<.01

* P<.05

REFERENCES

Aakvaag, A. 1969a. Steroid formation in porcine ovarian tissue in vitro. Acta. Endocrinol. 60:621.

Aakvaag, A. 1969b. Pathways in the biosynthesis of adrostenedione in the human ovary in vitro. Acta. Endocrinol. 60:517.

Abraham, G.E., R. Swerdloff, D. Tulchinsky and W.D. Odell. 1971. Radioimmunoassay of plasma progestin. J. Clin. Endocrinol. 32:619.

Akbar, A.M., L.E. Reichert, Jr., T.G. Dunn, C.C. Kaltenbach and G.C. Niswender. 1974. Serum levels of follicle-stimulating hormone during the bovine estrous cycle. J. Anim. Sci. 39:360.

Aitken, M.M. and J. Sanford. 1975. Effects of prostaglandins in calves. Br. J. Pharmacol. 54:266.

Änggård, E. and S. Bergström. 1963. Biological effects of an unsaturated trihydroxy acid ($PGF_{2\alpha}$) from normal swine lung. Acta. Physiol. Scand. 58:1.

Armstrong, D.T. 1968. Gonadotropins, ovarian metabolism, and steroid biosynthesis. Rec. Prog. Horm. Res. 24:255.

Baker, T.G., R.H.F. Hunter and P. Neal. 1975. Studies on the maintenance of porcine Graafian follicles in organ culture. Experientia 31:133.

Behrman, H.R., K. Yoshingaga and R.O. Greep. 1971. Extraluteal effects of prostaglandins. Ann. N.Y. Acad. Sci. 180:426.

Bellows, R.A., D.C. Anderson and R.E. Short. 1969. Dose-response relationships in synchronized beef heifers treated with follicle stimulating hormone. J. Ani. Sci. 28:638.

Bennett, A. and B. Fleshler. 1970. Prostaglandins and the gastro-intestinal tract. Gastroenterology 59:790.

Bjersing, L. and H. Carstensen. 1967. Biosynthesis of steroids by granulosa cells of the porcine ovary in vitro. J. Reprod. Fert. 14:101.

Brunner, M.A., L.E. Donaldson and W. Hansel. 1969. Exogenous hormones and luteal function in hysterectomized and intact heifers. J. Dairy Sci. 52:1849.

Caldwell, B.V., S.A. Tillson, W.A. Brock and L. Speroff. 1972. The effects of exogenous progesterone and estradiol on prostaglandin F levels in ovariectomized ewes. Prostaglandins 1:217.

Caldwell, B.V., F.J. Auletta and L. Speroff. 1973. Prostaglandins in the control of ovulation, corpus luteum function, and parturition. J. Reprod. Med. 10:133.

Capraro, D.L., M.W. Vernon, R.M. Abrams and D.C. Sharp. 1976. Effects of oxytocin and $PGF_{2\alpha}$ on mare uterine motility. J. Anim. Sci. 43:277.

Carsten, M.E. 1972. Prostaglandins part in regulating uterine contraction by transport of calcium. In E.M. Southern (Ed.) The Prostaglandins: Clinical Applications in Human Reproduction. Futura Publishing Co., Mount Kisco, N.Y.

Channing, C.P. 1970a. Influences of the in vivo and in vitro hormonal environment upon luteinization of granulosa cells in tissue culture. Rec. Prog. Horm. Res. 26:589.

Channing, C.P. 1970b. Effects of stage of the estrous cycle and gonadotropins upon luteinization of porcine granulosa cells in culture. Endocrinology 87:156.

Channing, C.P. 1974. Temporal effects of LH, hCG, FSH and dibutyryl cyclic 3', 5'-AMP upon luteinization of rhesus monkey granulosa cells in culture. Endocrinology 94:1215.

Channing, C.P. 1975. Follicle stimulating hormone stimulation of ^{125}I-human chorionic gonadotropin binding in porcine granulosa cell cultures. Proc. Soc. Exp. Biol. Med. 149:238.

Channing, C.P. and S.P. Coudert. 1976. Contribution of granulosa cells and follicular fluid to ovarian estrogen secretion in rhesus monkey in vivo. Endocrinology 98:590.

Channing, C.P. and S. Kammerman. 1973. Characteristics of gonadotropin receptors of porcine granulosa cells during follicular maturation. Endocrinology 92:531.

Channing, C.P. and S. Kammerman. 1974. Binding of gonadotropins to ovarian cells. Biol. Reprod. 10:179.

Chenault, J.R. 1973. Transitory changes in plasma progestins, estradiol and LH approaching ovulation and after prostaglandin $F_{2\alpha}$ injection in the bovine. M.S. Thesis, University of Florida, Gainesville.

Chenault, J.R., W.W. Thatcher, P.S. Kalra, R.M. Abrams and C.J. Wilcox. 1975. Transitory changes in plasma progestins, estradiol and luteinizing hormone approaching ovulation in the bovine. J. Dairy Sci. 58:709.

Chenault, J.R., W.W. Thatcher, P.S. Kalra, R.M. Abrams and C.J. Wilcox. 1976. Plasma progestins, estradiol and luteinizing hormone following prostaglandin $F_{2\alpha}$ injection. J. Dairy Sci. 59:1342.

Choudary, J.B., H.T. Gier and G.B. Marion. 1968. Cyclic changes in bovine vesicular follicles. J. Anim. Sci. 27:468.

Coceani, F., J.J. Dreifuss, L. Puglisi and L.S. Wolfe. 1969. Protaglandins and membrane function. In P. Mategazza and E.W. Horton (Eds.) Prostaglandins, Peptides and Amines. Academic Press, N.Y.

Cooper, M.J. 1974. Control of oestrous cycles of heifers with a synthetic prostaglandin analogue. Vet. Rec. 95:200.

Cooper, M.J. and P.S. Jackson. 1975. The application of cloprostenol (Compound ICI 80,996) in the control of the bovine oestrous cycle. Int. Conf. on Prostaglandins, Florence.

Coyotupa, J., A.F. Parlow and G.E. Abraham. 1972. Simultaneous radioimmunoassay of plasma testosterone and dihydrotestosterone. Analyt. Letters 5:239.

Dobson, H. 1973. The effect of HCG on follicular activity in the cow. J. Reprod. Fert. 35:605.

Dobson, H. and P.D.G. Dean. 1974. Radioimmunoassay of oestrone, oestradiol -17α and -17β in bovine plasma during the oestrous cycle and last stages of pregnancy. J. Endocrinol. 61:479.

Dorrington, J.H., Y.S. Moon and D.T. Armstrong. 1975. Estradiol -17β biosynthesis in cultured granulosa cells from hypophysectomized immature rats; stimulation by follicle-stimulating hormone. Endocrinology 97:1328.

DuCharme, D.W., J.R. Weeks and R.G. Montgomery. 1968. Studies on the mechanism of the hypertensive effect of prostaglandin $F_{2\alpha}$. J. Pharmacol. Exptl. Therap. 160:1.

El-Fouly, M.A., B. Cook. M. Nekola and A.V. Nalbandov. 1970. Role of the ovum in follicular luteinization. Endocrinology 87:288.

Ellicott, A.R., J.R. Scoggins, J.R. Hill, Jr., and D.M. Hendricks. 1974. Estrous control using $PGF_{2\alpha}$ I.LH and fertility. J. Anim. Sci. 39:207.

Eshkol, A. and B. Lunenfeld. 1972. Gonadotropic regulation of ovarian development in mice during infancy. In B.B. Savena, C.G. Beling and H.M. Gandy (Eds.) Gonadotropins. John Wiley and Sons, N.Y.

Falch, B. 1959. Site of production of estrogen in rat ovaries as studied in microtransplants. Acta. Physiol. Scand. 47, Suppl. 163.

Ferreira, S.H. and J.R. Vane. 1967. Prostaglandins: Their disappearance from and release into the circulation. Nature 216:868.

Ford, S.P., C.W. Weems, R.E. Pitts, J.E. Pexton, R.L. Butcher and E.K. Inskeep. 1975. Effects of estradiol -17β and progesterone on prostaglandins F in sheep uteri and uterine venous plasma. J. Anim. Sci. 41:1407

Fraioli, F., A. Fabbrini, V. Santiemma and G. Spera. 1972. Autoradiographic and spectrometric studies on the localization of 125-I labeled HCG, HLH, and FSH in immature rats. Folia Endocrin. 25:177.

Glencross, R.G., I.B. Munro, B.E. Senior and G.S. Pope. 1973. Concentrations of oestradiol -17β, oestrone and progesterone in jugular venous plasma of cows during the oestrous cycle and in early pregnancy. Acta. Encocrinol. 73:374.

Graves, N.W., T.G. Dunn, C.C. Kaltenbach, R.E. Short and J.B. Carr. 1975. Estrus and ovulation with $PGF_{2\alpha}$, SC21009 and GnRH. J. Anim. Sci. 41:354.

Gwazdauskas, F.C. 1974. Interrelationships of certain thermal and endocrine phenomena and reproductive performance in the female bovine. Ph.D. Dissertation, University of Florida, Gainesville.

Gwazdauskas, F.C., R.M. Abrams, W.W. Thatcher, F.W. Bazer and D. Baton. 1974. Thermal changes of the bovine uterus following administration of estradiol -17β. J. Anim. Sci. 39:87.

Hafs, H.D., T.M. Louis, P.A. Noden and W.D. Oxender. 1974. Control of the estrous cycle with prostaglandin $F_{2\alpha}$ in cattle and horses. J. Anim. Sci. 38:10, Suppl. I.

Hafs, H.D., J.G. Mans and G.E. Lamming. 1975. Fertility of cattle from AI after $PGF_{2\alpha}$. J. Anim. Sci. 41:355.

Hales, J.R.S., J.W. Bennett, J.A. Baird and A.A. Fawcett. 1973. Thermoregulatory effects of prostaglandins E_1, E_2, $F_{1\alpha}$, $F_{2\alpha}$ in the sheep. Pflugers Arch. Euro. J. Phy. 339:125.

Hall, P.F. and D.K. Young. 1968. Site of action of tropic hormones upon the biosynthetic pathways to steroid hormones. Endocrinology 82:559.

Heersche, G., G.H. Kiracufe, R.M. McKee, D.L. Davis and G.R. Brower. 1974. Control of estrus in heifers with $PGF_{2\alpha}$ and Synchro-Mate B. J. Anim. Sci. 38:225.

Henderson, K.M. and K.P. McNatty. 1975. A biochemical hypothesis to explain the mechanism of luteal regression. Prostaglandins 9:779.

Henricks, D.M., J.F. Dickey and G.D. Niswender. 1970. Serum luteinizing hormone and plasma progesterone levels during the estrous cycle and early pregnancy in cows. Biol. Reprod. 2:346.

Henricks, D.M., J.F. Dickey and J.R. Hill. 1971. Plasma estrogen and progesterone levels in cows prior to and during estrus. Endocrinology 89:1350.

Henricks, D.M., J.T. Long, J.R. Hill and J.F. Dickey. 1974. The effect of prostaglandin $F_{2\alpha}$ during various stages of the oestrous cycle of beef heifers. J. Reprod. Fert. 41:113.

Hisaw, F.L. 1947. Development of the Graafian follicle and ovulation. Physiol. Rev. 27:95.

Hixon, J.E., R. Nadaraja, R.J. Schechter and W. Hansel. 1973. Prostaglandin $F_{2\alpha}$-induced stimulation of estrone and estradiol -17β secretion in cattle. Prostaglandins 4:679.

Hobson, W.C. and W. Hansel. 1972. Plasma LH levels after ovariectomy, corpus luteum removal, and estradiol administration in cattle. Endocrinology 91:185.

Horton, E.W. and I.H.M. Main. 1964. A comparison of the biological activities of four prostaglandins. Brit. J. Pharmacol. Chemotherp. 21:182.

Horton, E.W. and I.H.M. Main. 1967. Further observations on the central nervous actions of prostaglandins $F_{2\alpha}$ and E_1. Brit. J. Pharmacol. Chemotherap. 30:568.

Hotchkiss, J., L.E. Atkinson and E. Knobil. 1971. Time course of serum estrogen and luteinizing hormone (LH) concentrations during the menstrual cycle of the rhesus monkey. Endocrinology 98:1971.

Inskeep, E.K. 1973. Potential uses of prostaglandins in control of reproductive cycles of domestic animals. J. Anim. Sci. 36:1149.

Kalra, P.S., W.W. Thatcher, M.M. Casey and C.J. Wilcox. 1974 Effect of GnRH in $PGF_{2\alpha}$ pretreated heifers. J. Anim. Sci. 39:214.

Karim, S.M.M., K. Hillier, K. Somers and R.R. Trussell. 1971. The effect of prostaglandins E_2 and $F_{2\alpha}$ administration by different routes on uterine activity and the cardiovascular system in pregnant and non-pregnant women. J. Obstet. Gyn. Brit. Comm. 78:172.

Kimball, F.A. and J.W. Lauderdale. 1975. Prostaglandin E_1 and $F_{2\alpha}$ specific binding in bovine corpora lutea: comparison with luteolytic effects. Prostaglandins 10:313.

Kimball, F.A., F. Bartol, W.W. Thatcher and F.W. Bazer. 1976. Unpublished observations.

Kraiem, Z. and L.T. Samuels. 1974. The influence of FSH and FSH + LH on steroidogenic enzymes in the immature mouse ovary. Endocrinology 95:660.

Lacroix, E., W. Eechaute and I. Leusen. 1974. The biosynthesis of estrogens by cow follicles. Steroids. 23:337.

Lambert, P.W., D.R. Griswold, V.A. LaVoie and E.L. Moudy. 1975. AI beef management with $PGF_{2\alpha}$ controlled estrus. J. Anim. Sci. 41:364.

Lauderdale, J.W. 1972. Effects of $PGF2_{\alpha}$ on pregnancy and estrous cycle of cattle. J. Anim. Sci. 35:246.

Lauderdale, J.W., B.E. Sequin, J.N. Stellflug, J.R. Chenault, W.W. Thatcher, C.K. Vincent and A.F. Loyancano. 1974. Fertility of cattle following $PGF_{2\alpha}$ injection. J. Anim. Sci. 38:964.

Lauderdale, J.W., J.D. Peuler, G.A. Johnson and P.A. Miller. 1975. Catecholamines and rectal temperature after $PGF_{2\alpha}$. J. Anim. Sci. 41:365.

LaVoie, V.A., G.R. Poncelet, D.K. Han, C.L. Soliday, P.W. Lambert and E.L. Moody. 1975. Effect of prostaglandin $F_{2\alpha}$ on the estrous cycle, corpora lutea and progesterone levels of hysterectomized cows. J. Anim. Sci. 41:166.

Lee, J.B., B.G. Covino, B.H. Takman and E.R. Smith. 1965. Renomedullary vasodepressor substance, medullin: Isolation, chemical characterization and physiological properties. Circ. Res. 17:57.

Lewis, A.J. and P. Eyre. 1972. Some cardiovascular and respiratory effects of prostaglandins E_1, E_2 and $F_{2\alpha}$ in the calf. Prostaglandins 2:55.

Lieberman, M.E., A. Barnea, S. Bauminger, A. Tsafriri, W.P. Collins and H.R. Linder. 1975. LH effect on the pattern of steroidogenesis in cultured Graafian follicles of the rat: Dependence on macromolecular synthesis. Endocrinology 96:1533.

Linder, H.R., A. Tsafriri, M.E. Lieberman, U. Zor, Y. Koch, S. Bauminger and A. Barnea. 1974. Gonadotropin action on cultured Graafian follicles: Induction of maturation division of the mammalian oocyte and differentiation of the luteal cell. Rec. Prog. Horm. Res. 30:79.

Lostroh, A.J. and R.E. Johnson. 1966. Amounts of interstitial cell-stimulating hormone and follicle stimulating hormone required for follicular development, uterine growth and ovulation in the hypophysectomized rat. Endocrinology 79:991.

Louis, T.M., H.D. Hafs and B.E. Sequin. 1973. Progesterone, LH, estrus and ovulation after prostaglandin $F_{2\alpha}$ in heifers. Proc. Soc. Exp. Biol. Med. 143:152.

Louis, T.M., H.D. Hafs and D.A. Morrow. 1974a. Intrauterine administration of prostaglandin $F_{2\alpha}$ in cows: Progesterone, estrogen, LH, estrus and ovulation. J. Anim. Sci. 38:347.

Louis, T.M., J.N. Stellflug, H.A. Tucker and H.D. Hafs. 1974b. Plasma prolactin, growth hormone, luteinizing hormone and glucocorticoids after prostaglandin $F_{2\alpha}$ in heifers. Proc. Soc. Exp. Biol. Med. 147:128.

Louis, T.M., J.N. Stellflug and H.D. Hafs. 1975. Blood LH during luteolysis induced by $PGF_{2\alpha}$. J. Anim. Sci. 41:366.

McCracken, J.A., D.T. Baird and J.R. Goding. 1971. Factors affecting the secretion of steroids from the transplanted ovary in the sheep. Rec. Prog. Horm. Res. 27:537.

McIntosh, J.E.A. and R.M. Moor. 1973. Regulation of steroid secretion in sheep ovarian follicles. J. Reprod. Fert. 35:605.

Main, I.H.M. 1973. Prostaglandins and the gastro-intestinal tract. In M.F. Cuthbert (Ed.) The Prostaglandins. J.B. Lippincott Co., Philadelphia.

Makris, A. and K.J. Ryan. 1975. Progesterone, androstenedione, testosterone, estrone and estradiol synthesis in hamster ovarian follicle cells. Endocrinology 96:694.

Marion G.B., H.T. Gier and J.B. Choudary. 1968. Micromorphology of the bovine ovarian follicular system. J. Anim. Sci. 27:451.

Mark, A.L., P.G. Schmid, J.W. Eckstein and M.G. Wendling. 1971. Venous responses to prostaglandin $F_{2\alpha}$. Amer. J. Phy. 220:222.

Midgley, A.R., Jr. 1973. Autoradiographic analysis of gonadotropin binding to rat ovarian tissue sections. Adv. Exp. Med. Biol. 36:365.

Miller, P.A., J.W. Lauderdale and S. Geng. 1976. Effects of various doses of Prostin F_2 alpha on estrous cycles, rectal temperature, sweating, heart rate and respiration rate in mares. J. Anim. Sci. 42:901.

Mills, T.M. and K. Savard. 1972. In vitro steriod synthesis by follicles isolated from the rabbit ovary. Steroids 20:247.

Mills, T.M. and K. Savard. 1973. Steroidogenesis in ovarian follicles isolated from rabbits before and after mating. Endocrinology 92:788.

Moon, Y.S., J.H. Dorrington and D.T. Armstrong. 1975. Stimulatory action of follicle-stimulating hormone on estradiol -17β secretion by hypophysectomized rat ovaries in organ culture. Endocrinology 97:244.

Moor, R.M. 1973. Oestrogen production by individual follicles explanted from ovaries of sheep. J. Reprod. Fert. 32:545.

Moor, R.M. 1974. The ovarian follicle of the sheep: inhibition of oestrogen secretion by luteinizing hormone. J. Endocrin. 61:455.

Moor, R.M., M.F. Hay, J.E.A. McIntosh and B.V. Caldwell. 1973. Effect of gonadotrophins on the production of steroids by sheep ovarion follicles cultured in vitro. J. Endocrin. 58:599.

Moor, R.M., M.F. Hay and R.F. Seamark. 1975. The sheep ovary: regulation of steroidogenic, haemodynamic and structural changes in the largest follicle and adjacent tissue before ovulation. J. Reprod. Fert. 45:595.

Nakano, J. and B. Cole. 1969. Effects of prostaglandins E_1 and $F_{2\alpha}$ on systemic pulmonary and splanchnic circulations in dogs. Am. J. Phy. 217:222.

Nakano, J. and J.R. McCurdy. 1968. Hemodynamic effects of prostaglandins E_1, A_1 and $F_{2\alpha}$ in dogs. Proc. Soc. Exptl. Biol. Med. 128:39.

Nakano, J., S. Chiba and T. Nakajima. 1971. Effect of prostaglandins E_1 and $F_{2\alpha}$ on heart rate by direct injection into the canine sinus node artery. Clin. Res. 19:645.

Nekola, M.V. and A.V. Nalbandov. 1971. Morphological changes of rat follicular cells as influenced by oocytes. Biol. Reprod. 4:154.

Novy, M.J. and M.J. Cook. 1973. Redistribution of blood flow by prostaglandin $F_{2\alpha}$ in the rabbit ovary. Am. J. Obstet. Gynecol. 117:381.

Oakey, R.E. and S.R. Stitch. 1968. Oestrogen biosynthesis by segments of bovine follicle and the effect of follicle stimulating hormone preparations. Acta. Endocrin. 58:407.

Patwordhan, V.V. and A. Lanthier. 1971. The in vitro formation of androstenedione from C_{21} steroids by follicles isolated from human ovaries. Acta. Endocrin. 67:698.

Pharriss, B.B. and L.J. Wyngarden. 1969. The effect of prostaglandin $F_{2\alpha}$ on the progestogen content of ovaries from pseudopregnant rats. Proc. Soc. Exp. Biol. Med. 130:92.

Priedkalns, J. and A.F. Weber. 1968. Ultrastructural studies of the bovine Graafian follicle and corpus luteus. Z. Zellforsch Mikrosk. Anat. 91:554.

Rajakoski, E. 1960. The ovarian follicular system in sexually mature heifers with special reference to seasonal, cyclical and left-right variations. Acta. Endocrinol. Suppl. 52:34.

Rajaniemi, M. and T. Vanha-Pertulla. 1972. Specific receptor for LH in the ovary. Evidence by auto-radiography and tissue fractionation. Endocrinology 90:1.

Rao, C.V. 1975. The presence of discrete receptors for prostaglandin $F_{2\alpha}$ in the cell membranes of bovine corpora lutea. Biochem. Biophy. Res. Comm. 64:416.

Raz, A. 1972. Interaction of prostaglandins with blood plasma proteins. III. Rate of dissappearance and metabolites formation after intravenous administration of free or albumin bound prostaglandin $F_{2\alpha}$ and A_2. Life Sci. 11:965.

Rexroad, C.E. and C.R. Barb. 1975. Uterine response of estrous ewes to prostaglandin. J. Anim. Sci. 41:376.

Richards, J.S. and A.R. Midgley, Jr. 1976. Protein hormone action: a key to understanding ovarian follicular and luteal cell development. Biol. Reprod. 14:82.

Roche, J.F. 1976a. Fertility in cows after treatment with a prostaglandin analogue with or without progesterone. J. Reprod. Fert. 46:341.

Roche, J.F. 1976b. Synchronization of oestrus in cattle. World Rev. Anim. Prod. 12:79.

Rodriguez, T.R., M.J. Fields, W.C. Burns, D.E. Franke, J.F. Hentges, W.W. Thatcher and A.C. Warnick. 1975. Breeding at a predetermined time in the bovine following $PGF_{2\alpha}$ + GnRH. J. Anim. Sci. 40:188.

Roman-Ponce, H. 1977. Ph.D. Dissertation, University of Florida, Gainesville.

Roman-Ponce, H., D. Caton, D.H. Barron, W.W. Thatcher and C.J. Wilcox. 1976. Thermal stress effects on uterine blood flow. J. Anim. Sci. 43:301.

Roman-Ponce, H., W.W. Thatcher, D. Caton, D.H. Barron and C.J. Wilcox. 1977. Thermal stress effects on uterine blood flow in sheep. J. Anim. Sci. In press.

Ryan, K.J. and R.V. Short. 1965. Formation of estradiol by granulosa and thecal cells of the equine ovarian follicle. Endocrinology 76:108.

Ryan, K.J. and O.W. Smith. 1965. Biogenesis of steroid hormones in the human ovary. Rec. Prog. Horm. Res. 21:367.

Ryan, K.J., Z. Petro and J. Kaiser. 1968. Steroid formation by isolated and recombined ovarian ganulosa and thecal cells. J. Clin. Endocrinol. 28:355.

Savard, K., J.M. Marsh and B.F. Rice. 1965. Gonadotropins and ovarian steroidogenesis. Rec. Prog. Horm. Res. 21:285.

Seamark, R.F., R.M. Moor and J.E.A. McIntosh. 1974. Steroid hormone production by sheep ovarian follicles cultured in vitro. J. Reprod. Fert. 41:143.

Shemesh, M.G., N. Ayalon and H.R. Linder. 1972. Oestradiol levels in the peripheral blood of cows during the estrous cycle. J. Endocrinol. 55:72.

Shemesh, M. and W. Hansel. 1975a. Stimulation of follicular steroidogenesis by $PGF_{2\alpha}$. J. Anim. Sci. 41:379.

Shemesh, M. and W. Hansel. 1975b. Levels of prostaglandin F(PGF) in bovine endometrium, uterine venous, ovarian arterial and jugular plasma during the estrus cycle. Proc. Soc. Expt. Biol. Med. 148:123.

Short, R.V. 1962a. Steroids in the follicular fluid and the corpus luteum of the mare. A 'two cell type' theory of ovarian steroid synthesis. J. Endocrinol. 24:59.

Short, R.V. 1962b. Steroid concentration in normal follicular fluid and ovarian cyst fluid from cows. J. Reprod. Fert. 4:27.

Short, R.V., B.E. Howland, R.D. Randel, D.S. Christensen and R.A. Bellows. 1973. Induced LH release in spayed cows. J. Anim. Sci. 37:551.

Snedecor, G.W. and W.G. Cochran. 1967. Statistical Methods. The Iowa State University Press, Ames.

Snook, R.B., R.R. Saatman and W. Hansel. 1971. Serum progesterone and luteinizing hormone levels during the bovine estrous cycle. Endocrinology 88:678.

Stacy, B.D., R.T. Gremmell and G.D. Thorburn. 1976. Morphology of the corpus luteum in the sheep during regression induced by prostaglandin $F_{2\alpha}$. Biol. Reprod. 14:280.

Steelman, S.L. and F.M. Pohley. 1953. Assay of the follicle stimulating hormone based on the augmentation with human chorionic gondotropin. Endocrinology 53:604.

Stellflug, J.N., T.M. Louis, R.C. Gorewit, W.D. Oxender and H.D. Hafs. 1975. Luteolysis after $PGF_{2\alpha}$ in hysterectomized cattle. J. Anim. Sci. 41:38.

Thatcher, W.W. and J.R. Chenault. 1976. Reproductive physiological responses of cattle to exogenous prostaglandin $F_{2\alpha}$. J. Dairy Sci. 59:1366.

Thorburn, G.D. and J.R.S. Hales. 1972. Selective reduction in blood flow to the ovine corpus luteum after infusion of prostaglandin $F_{2\alpha}$ into a uterine vein. Abst. Proc. Austral. Phys. & Phar. Soc. 3:145.

Tobey, D.M. and W. Hansel. 1975. Insemination at a predetermined time after prostaglandin $F_{2\alpha}$, estradiol and luteinizing hormone releasing hormone treatments. J. Dairy Sci. 58:769.

Umo, I. 1975. Effect of Prostaglandin $F_{2\alpha}$ on the ultrastructure and function of sheep corpora lutea. J. Reprod. Fert. 43:287.

Vanasin, B., W. Greenhough and M.M. Schuster. 1970. Effect of prostaglandin on electrical and motor activity of isolated colonic muscle. Gastroenterology 58:1004.

Viguera, M.G. and F.A. Sunahara. 1969. Microcirculatory effects of prostaglandins. Can. J. Phy. Pharm. 47: 627.

Weiner, R. and G. Kaley. 1972. Lysosomal fragility induced by prostaglandin $F_{2\alpha}$. Nature New Biol. 236:46.

Weiss, T.J., R.F. Seamark, J.E.A. McIntosh and R.M. Moor. 1976. Cyclic AMP in sheep ovarian follicles: site of production and response to gonadotrophins. J. Reprod. Fert. 46:347.

Welch, J.A., A.J. Hackett, C.J. Cunningham, J.O. Heishman, S.P. Ford, R. Nadaraja, W. Hansel and E.K. Inskeep. 1975. Control of estrus in lactating beef cows with prostaglandin $F_{2\alpha}$ and estradiol benzoate. J. Anim. Sci. 41:1686.

Wettemann, R.P., H.D. Hafs, L.A. Edgerton and L.V. Swanson. 1972. Estradiol and progesterone in blood serum during the bovine estrous cycle. J. Anim. Sci. 34: 1020.

YoungLai, E.V. 1972. The influence of follicular fluid and plasma on the steroidogenic activity of equine granulosa cells. J. Reprod. Fert. 28:95.

YoungLai, E.V. 1973. The bioconversion of androstenedione and testosterone by rabbit granulosa cells and the effect of ovine LH on thecal aromatization. Steroids Lipids Res. 4:11.

YoungLai, E.V. 1974a. Steroid production by the isolated rabbit ovarian follicle. I. Effects of ovine LH. J. Reprod. Fert. 40:95.

YoungLai, E.V. 1974b. Steroid production by the isolated rabbit ovarian follicle. II. Effect of peptid hormones. Steroids Lipids Res. 5:49.

YoungLai, E.V. 1975a. Steroid production by the isolated rabbit ovarian follicle. 1975. J. Reprod. Fert. 45:575.

YoungLai, E.V. 1975b. Steroid production by the isolated rabbit ovarian follicle. III. Actinomycin D-insensitive stimulation of steroidogenesis by LH. Endocrinology 96:468.

YoungLai, E.V. and R.V. Short. 1970. Pathways of steroid biosynthesis in the intact Graafian follicle of mares in oestrus. J. Endocrinol. 47:321.

Zeleznik, A.J., A.R. Midgley, Jr., and L.E. Reichert, Jr. 1974. Granulosa cell maturation in the rat: Increased binding of human chorionic gonadotropin following treatment with follicle-stimulating hormone in vivo. Endocrinology 95:818.

Zolman, J., E.M. Convey, J.H. Britt and H.D. Hafs. 1973. Release of bovine luteinizing hormone by purified porcine and synthetic gonadotropin releasing hormone. Proc. Soc. Exp. Biol. Med. 142:189.

BIOGRAPHICAL SKETCH

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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